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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

V. ON THE INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON DURATION OF LIFE IN DROSOPHILA[1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

A. INFLUENCE OF VENTILATION ON DURATION OF LIFE

THE standard method of handling *Drosophila* cultures, as described by Pearl and Parker (27), includes the plugging of the mouth of the bottle with absorbent cotton to prevent the escape of the flies. The theory of this practice, which is the custom in Morgan's laboratory, presumably is that air will pass in and out through the plug while the flies can not. No physicist or ventilation engineer would, we believe, accept this theory. Many years ago the senior author had occasion to make some observations on the ventilation of curtain-front poultry houses, and soon came to the conclusion that curtains of one thickness only, of the very porous jute bagging which is used for bran sacks, are practically nearly as effective in *preventing* the natural unforced circulation of air as a half-inch pine board would be. We may be sure that the plug of cotton used in *Drosophila* bottles will be an even more certain preventative of the natural unforced circulation of air. Theoretically one may perhaps hold that there is *more* circulation of air with a cotton plug than there would be with a cork stopper, but the difference must be infinitesimal.

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 67.

In the systematic survey which we are making, at the beginning of our experimental study of duration of life, it seemed desirable to test the influence upon this character of degree of natural ventilation of the bottles. It is the purpose of this first section of this paper to present the results of some experiments on this point.

Material and Methods

The experiments were carried out in two series. For the first, wild type flies of our Old Falmouth stock (Pearl and Parker (27)) belonging to Line 107, the duration of life constants of which have been given by Pearl and Parker (32), were used. These flies were of the 25th pedigreed generation. Eighteen mass matings of the flies of this line were started for the present experiments on March 13, 1922, and the flies to be used emerged March 23–30, 1922.

For the second series short-lived flies of Quintuple stock (Pearl and Parker (27)) were used, in the 27th pedigreed generation. Twenty-five mass matings of Quintuple line 405 were started April 10, 1922, and six mass matings of mixed Quintuple stock were started April 11, 1922. The flies for use in the experiment emerged April 22–27, 1922.

The procedure in making up the experiments was as follows: The flies were counted out each morning upon emergence into our standard one ounce screw top shell vials used in the determination of duration of life (Pearl and Parker (27)). Fifty flies were put in each bottle. The wild type flies were counted in through the counting tube described in these Studies, III (Pearl and Parker (44)). The Quintuple flies move through the tube so slowly, however, that there is time for moisture to condense and accumulate on the walls of the tube, killing some of the flies by drowning, and injuring others. Consequently the flies of this type were etherized and counted into the bottles. It has been shown in these Studies, III, that such etherization has no measurable

influence on duration of life. Each day's flies were divided equally between control and experimental groups. Fertility in the Quintuple flies is so low that even with the large number of matings the hatches on some days did not equal 100 flies. It thus resulted that there were a few bottles of the Quintuples with fewer than 50 flies to the bottle: 1 case with 40 flies in control and 40 in the experimental bottle, 1 with 37 flies in each bottle, and 1 with 23 flies in each bottle.

The *control* bottles were plugged with cotton in the ordinary way. The experimental, ventilated bottles were covered with one layer of silk bolting cloth of No. 48 mesh (48 meshes to the linear inch), this being the largest mesh which could be used without any possibility of a fly squeezing through the openings. The cloth was held firmly and evenly in place by an aluminum screw cap in the top of which a central hole a little more than ¾ inch in diameter had been punched out. This is practically the internal diameter of the shell vials which we use.

Both control and ventilated bottles were carried in 25° incubators, and all other procedure was that which has been described by the authors (27) as standard in the work of this laboratory on duration of life in *Drosophila*.

Results

The observed l_x distributions (survivors out of 1,000 starting together) for the wild type Old Falmouth Line 107 are given in Table I, together with the absolute numbers of flies on which the distributions are based.

These distributions of Table I are shown graphically in Fig. 1.

It is evident at once that the flies in the well-ventilated bottles outlive those in the ill-ventilated. Their expectation of life is greater at every age. The magnitude and significance of this difference can best be appreciated from the constants of duration of life set forth in Table II.

TABLE I

SURVIVORSHIP DISTRIBUTIONS (l_x) OF VENTILATED AND CONTROL FLIES
Old Falmouth, Line 107

Age in days	Number of survivors up to indicated age. Ventilated	Control
1	1,000	1,000
7	986	984
13	970	969
19	946	929
25	900	846
31	804	730
37	697	615
43	589	485
49	480	397
55	371	292
61	278	195
67	185	119
73	103	44
79	12	2
85	0	0
Absolute number of flies	946	931

TABLE II

FREQUENCY CONSTANTS OF d_x DISTRIBUTIONS
Wild Type, Line 107

Group	Mean	Standard Deviation	Coefficient of Variation
Control	43.66 ± .39	17.63 ± .28	40.38 ± .73
Ventilated	47.92 ± .40	18.22 ± .28	38.01 ± .67
Difference	+ 4.26 ± .56	+ .59 ± .39	− 2.37 ± .99

There is clearly a significant increase, amounting roughly to 10 per cent., in the mean duration of life, or expectation of life at emergence, in the flies in the ventilated as compared with the unventilated bottles, all other conditions both genetic and environmental having been the same in the two series.

That the increased amount of fresh air is the cause of the difference is evidenced by the behavior of the flies. In the ventilated bottles the flies tended at all times to congregate on the under side of the bolting cloth going down to the bottom for food occasionally, but otherwise exhibiting a strong preference for the region about the

mouth of the bottle, where the diffusion of air between inside and outside was going on most rapidly. This behavior is in no way characteristic, in our experience, of flies in the bottles stoppered with cotton. In those there

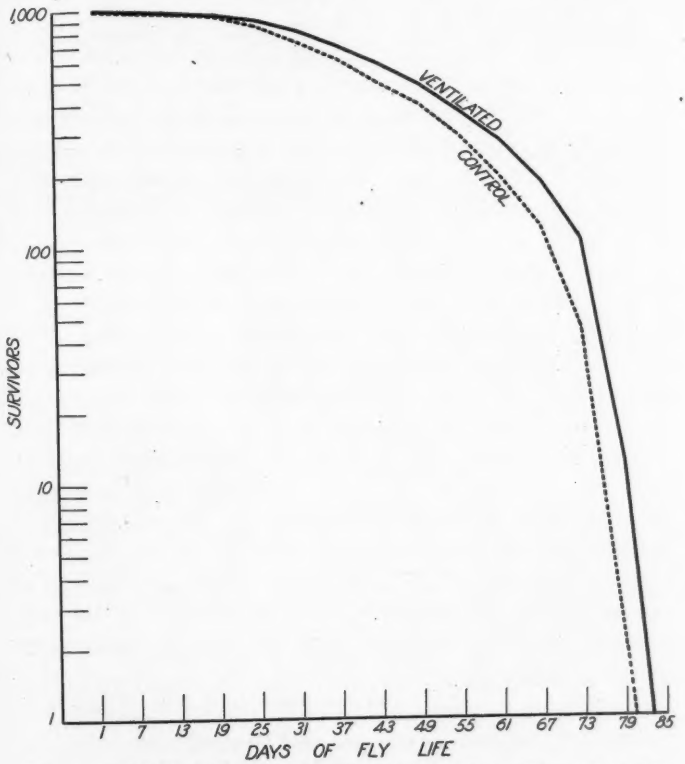

FIG. 1. Survivorship (l_x) lines for ventilated (solid line) and control (broken line) flies.

is generally a fairly even distribution of flies throughout the bottle, with such tendency towards concentration as there is, in the direction of the bottom near the food rather than the top.

In Table III are presented the survivorship distributions for the Quintuple flies. Because of their much shorter life span, as shown in the life tables of the first

one of the Studies (27), a shorter abscissal interval has been used in the grouping. The figures for Quintuple stock flies, and for an inbred Quintuple line (No. 405) are given separately.

TABLE III

SURVIVORSHIP DISTRIBUTIONS (l_x) OF VENTILATED AND CONTROL FLIES
Quintuple Stock and Line 405

Age in Days	Number of Survivors up to Indicated Age in			
	Stock Ventilated	Stock Control	Line 405 Ventilated	Line 405 Control
1	1,000	1,000	1,000	1,000
4	993	992	783	878
7	816	812	422	443
10	213	368	62	90
13	81	158	18	36
16	44	143	9	14
19	29	113	9	5
22	22	83	4	0
25	22	60	4	—
28	22	30	0	—
31	22	23	—	—
34	15	23	—	—
37	0	15	—	—
40	—	15	—	—
43	—	8	—	—
46	—	8	—	—
49	—	8	—	—
52	—	0	—	—
Absolute number of flies	136	133	209	221

The calculated constants from the d_x distributions are given in Table IV.

TABLE IV

FREQUENCY CONSTANTS OF d_x DISTRIBUTIONS
Quintuple Stock and Quintuple Line 405

Sort	Group	Mean	Standard Deviation	Coefficient of Variation
Quintuple stock	Control	11.07±.41	7.04±.29	63.58±3.54
	Ventilated	9.34±.26	4.57±.19	48.92±2.43
Difference		− 1.73±.49	−2.47±.35	−14.66±4.29
Quintuple (line 405)	Control	6.90±.13	2.90±.09	42.09±1.57
	Ventilated	6.78±.14	2.99±.10	44.06±1.71
Difference		− .12±.19	+ .09±.13	+ 1.97±2.32

The situation here is evidently quite different from what obtained with the wild type flies. The Quintuples lived somewhat longer in the control bottles than in the ventilated. In the case of flies from stock, the difference in the means amounts to 1.73 days, and is 3.5 times its probable error. The numbers are, however, small, and as an examination of Table III shows, the long survival of 2 individuals in the control series after age 34 accounts for a considerable part of the difference in the means. With a larger experimental sample much of the difference in the means would, we feel sure, disappear. The influence of these same two individual flies is clearly seen in the greatly increased variability of the control series over the ventilated in the stock groups.

In general we are of the opinion that in the case of Quintuple flies the difference in ventilation represented by a bolting cloth screen versus a cotton stopper has no significant influence upon duration of life. The results with extremely short-lived line 405, we regard as typical of what one should expect with Quintuple flies in this sort of an experiment.

The reason for the difference between wild type and Quintuple flies in their response to ventilation is founded, in our opinion, upon the normal differences in behavior between the two types. In Quintuples the wings do not function (the wing mutation in this stock is Vestigial). The consequence is that these flies are much less active, and generally appear to live on a lower metabolic plane, than wild type flies. Their oxygen needs are presumably smaller, and it would therefore be reasonable to expect that they would not show the difference in duration of life with increased ventilation that the wild type flies do. In this connection, it should be noted that their actual behavior in this experiment was in accordance with the view here suggested. They showed no such definite tendency to congregate at the top of the bottle under the bolting cloth as the wild type flies did. Their distribution was about the same in ventilated as in the control

bottles. Another consideration is that genetically Quintuple carries factors for very short life. These genes appear, in our experience with these flies, to be the overwhelmingly important factors in determining their length of life. No environmental factor, however favorable, makes much difference in their duration of life.

Summary

In experiments involving the determination of the duration of life in 2,576 individual flies, it has been found that in the case of *Drosophila* of wild type (*i.e.*, carrying no mutations so far as known), an increase of roughly 10 per cent. in the mean duration of life is brought about by increasing the ventilation of the culture bottles, by covering the mouth with one layer of No. 48 mesh bolting cloth, as compared with the use of cotton plug stoppers as is the usual practice in the culture of *Drosophila* in the laboratory. Owing, in our opinion, to fundamental differences in behavior, no such difference appears in the case of Quintuple flies.

B. Can the Duration of Life be Increased by Embryonic Juice?

If the theory of senescence and natural death which the senior author has developed in his "Biology of Death" (1–7) is true, one consequence of it should be that it might be possible to increase the duration of life, if by appropriate means one could restore the normal functional balance of the parts of the body after changes had set in with advancing age. Might it not be possible, by the use of X-rays for example, at the right stage of the life curve, and in proper dosage, to destroy cells, or perhaps even parts of tissues, which have got out of proper functional balance, and thus pave the way to their replacement by regeneration with fresh, "young" cells or tissues? In this way the life of the whole organism might be prolonged. The work of Frisch and Starlinger (45) with blood suggests that such a result might at least

be hoped for. In view of the known facts as to the potential immortality of tissue cells in cultures *in vitro,* and the apparent reason for the difference in the behavior of the same cells in respect of duration of life when they are in the multicellular body, all of which has been rather fully discussed by Pearl in the "Biology of Death" (*loc. cit.*), it would seem that this is a line of experimentation well worth following. We have a number of experiments along this line now in progress, particularly with X-rays, which we expect later to report upon. Some of the purely preliminary work has already been finished, and we wish in this paper to report one piece of it.

The brilliant researches of Carrel and his coworker Ebeling (*cf.* 46, 47) on the duration of life of cells in cultures *in vitro* have brought to light the extraordinarily interesting and presumably important fact that for the continued life of such cultures it is apparently essential to have in the culture medium a small amount of embryonic juice. In just what manner this functions is not yet clear, but the necessity of its presence seems well established.

It occurred to us in our preliminary work on prolongation of life in *Drosophila,* or as it is perhaps better to put it, on changing the form of the l_x line of the *Drosophila* life table, to see whether embryonic juice, applied at a point on the l_x curve after senescence had definitely set in, would have any effect upon the subsequent course of the curve, or in other words, upon the duration of life of the organism as a whole, comparable to its effect upon the life of cells in culture. The ideal way, of course, in such an experiment would be to get the embryonic juice to the tissues of the fly by a par-enteral route, but as no practical method of doing this occurred to us, we decided to feed it, and see if any results followed.

Material and Methods

The flies used in the experiment were all wild type, of Old Falmouth stock, and belonged to Line 107, pedigree

bred for 21 generations. The individuals for the experiment came from 20 mass matings of 3 pairs of parents each from this line. The bottles were started December 16, 1921, and the flies used in the experiment emerged December 28, 1921, to January 9, 1922.

The flies were counted through the counting tube into our standard shell-vials in groups of 50 each. Each day's bottles were divided into three groups at the beginning of the experiment, but all had the same regular treatment until the flies in them were 30 days old. This is a point where the l_x line is beginning distinctly to turn downward. From that time on until the end of their life one series of flies was given chicken juice in their food, and one series the juice and pulp of crushed *Drosophila* larvæ. The chick embryos used were 14 days old. The juice was extracted in a beef-juice extractor, and added to the regular food at the rate of approximately 2 c.c. to 100 c.c. of food. With the *Drosophila* larvæ, the whole pulp was used, and that too was added to the regular food at the rate of approximately 2 c.c. to each 100 c.c. of food. All the flies, experimental and control, were transferred every day to fresh food, made up that day, except on Sundays.

On Feb. 8 an accident happened to the incubator at the source of our chicken supply,[2] so that for 12 days no chickens were obtainable.

In all particulars except those specified above, the procedure in these experiments was the standard technique of this laboratory in duration of life work described in (27).

Results

The survivorship distributions are given in Table V, on the basis, *A*, of 1,000 starting at emergence, and, *B*, of 1,000 starting at age 31 days, that is at the time when the experimental feeding began.

[2] We are greatly indebted to our colleagues, Dr. and Mrs. Warren H. Lewis, for furnishing us with chicken material for this work.

TABLE V

SURVIVORSHIP DISTRIBUTIONS (l_x) OF GROUPS OF DROSOPHILA FED IN DIFFERENT WAYS

Old Falmouth Stock, Line 107

Age in Days	Number of Survivors up to Indicated Age in					
	Controls		Chicken Juice		Larval Pulp	
	A	*B*	*A*	*B*	*A*	*B*
1	1,000	—	1,000	—	1,000	—
7	975	—	970	—	970	—
13	945	—	935	—	930	—
19	884	—	873	—	858	—
25	797	—	784	—	719	—
31	616	1,000	648	1,000	591	1,000
37	491	796	456	703	464	785
43	362	588	313	484	283	479
49	286	465	246	380	210	356
55	212	345	198	306	156	264
61	156	253	152	234	116	196
67	120	197	110	170	82	140
73	59	96	58	89	46	78
79	21	34	24	38	28	48
85	7	11	9	14	3	5
91	1	2	0	0	1	2
97	1	2	—	—	0	0
103	0	0	—	—	—	—
Absolute number of flies	1,013	—	983	—	994	—

The biometric constants of duration of life calculated from the d_x distributions are given in Table VI.

TABLE VI

BIOMETRIC CONSTANTS FOR DURATION OF LIFE IN DROSOPHILA UNDER DIFFERENT CONDITIONS OF FOOD. *A.* FROM EMERGENCE.

B. FROM AGE 31 DAYS ON

Class	Group	Mean (in days)	Standard Deviation (in days)	Coefficient of Variation
A	Control	39.60±.40	18.76±.28	47.38± .85
	Chicken juice	38.66±.40	18.61±.28	48.12± .89
	Larval pulp	36.74±.39	18.01±.27	49.03± .90
B	Control	19.72±.40	14.66±.28	74.35±1.62
	Chicken juice	17.51±.40	15.05±.27	85.96±2.06
	Larval pulp	17.12±.39	14.03±.28	81.98±1.94

It is evident that there are no large differences in mean duration of life between any of the groups. The l_x distributions and the constants are closely similar throughout. This is true whether the whole life is taken, or the expectation after age 31. The exact nature of the differences is shown in Table VII.

TABLE VII

DIFFERENCES IN MEANS OF TABLE VI

Class	Difference Taken	Value of Difference	Diff./P.E. Diff.
A.....	Control – chicken juice.....	.94±.56	1.66
	Control – larval pulp.....	2.86±.55	5.15
	Chicken juice – larval pulp.....	1.92±.56	3.46
B.....	Control – chicken juice.....	2.21±.56	3.93
	Control – larval pulp.....	2.60±.56	4.67
	Chicken juice – larval pulp.....	.39±.56	.69

The control group had slightly the greatest duration of life, both as a whole, and from the time of the beginning of the special feeding on. The flies fed larval pulp had the worst expectation of life, with those fed chicken juice in an intermediate position. None of the differences, however, is large. That some of them are significant statistically probably means no more than that the changed food is not quite so favorable for the flies as the normal, standard food. The numbers involved are large relatively, and the probable errors consequently small.

We must then conclude that the administration of embryonic juice in the manner, amount and time in the life cycle, which defined its administration in these experiments, does not bring about any prolongation of the life of the whole organism, comparable to its effect in tissue cultures *in vitro*. This does not necessarily mean that under other conditions of administration or dosage an effect in this sense might not be produced. We believe, however, that it is not probable that any prolongation of life can be brought about by this method, for the reason that in the first place the results of the present ex-

periment give no suggestion that with larger dosage any such result would appear, and in the second place, because the experiments of Bacot and Harden (48) indicate that as slight (or slighter) alterations of the food of *Drosophila* as those of the present experiments may produce marked effects in respect of viability.[3]

For some reason which we are unable to explain, the flies of Line 107 had, in all the series of this experiment, a lower mean duration of life than this line has ever shown before (*cf.* 32, 44, and section *A* of the present paper). The values are extremely even and consistent in this feeding experiment, but are about 10 days lower than what previous work has indicated as the normal duration of life in this line. There has been no other change in the line, in fertility or other characters. We are inclined to believe that the low values in the present experiments represent merely a temporary secular change (? seasonal) in the duration of life characteristic of the line.

Summary

In experiments involving the determination of the duration of life in 2,990 individual flies, it was found that there was no prolongation of the life of *Drosophila* produced by adding embryonic juice (either from the chick, or from the larvæ of *Drosophila* itself) daily to the food, to the amount of 2 per cent. of the total food material, beginning with the 31st day of the flies' life.

LITERATURE CITED

(The plan of numbering citations is explained in the second of these Studies, AMER. NAT., Vol. 56, p. 174.)

44. Pearl, R. and Parker, S. L. Experimental Studies on the Duration of Life. III. The Effect of Successive Etherizations on the Duration of Life of *Drosophila*. AMER. NAT., Vol. 56, pp. 273–280, 1922.

45. Frisch, A. and Starlinger, W. Zur Frage der Protoplasma-activierung. *Zeitschr. f. d. ges. exp. Med.*, Bd. 24, pp. 142–158, 1921.

[3] It ought, however, to be pointed out that the experiments of Bacot and Harden are extremely faulty from a technical standpoint. They evidently know little of the practical husbandry of *Drosophila*. Their cultures were incubated at 30° C. At this temperature one does not get anything remotely resembling normal physiological processes or duration of life, except after many months of acclimatization.

46. Carrel, A. and Ebeling, A. H. The Multiplication of Fibroblasts *in vitro*. *Jour. Exp. Med.*, Vol. 34, pp. 317–337, 1921.
47. *Id.* Heterogenic Serum, Age, and Multiplication of Fibroblasts. *Ibid.*, Vol. 35, pp. 17–38, 1922.
48. Bacot, A. W. and Harden, A. Vitamin Requirements of *Drosophila*. I. Vitamines B and C. *Biochem. Jour.*, Vol. 16, pp. 148–152, 1922.

VI. A COMPARISON OF THE LAWS OF MORTALITY IN DROSOPHILA AND IN MAN

PROFESSOR RAYMOND PEARL

I

IN the first of these Studies (27) there were presented for the first time, so far as I am aware, complete life tables for any other organism than man. Up to the present time there have been presented in the published results of the work of this laboratory on *Drosophila* (27, 32, 44, 49, 50) exact determinations of the duration of life in 24,329 individual flies. This is a statistically respectable mass of material, and warrants some general discussion.

In the first study a rough, purely graphical comparison of the l_x lines of the *Drosophila* and certain human life tables was instituted. This comparison, rough as is was, made apparent at once the fact that there was a fundamental similarity in laws of mortality in these two organisms.

It is my purpose in the present paper to make a more exact comparison of the values of the life table functions in the two cases. It will be seen that the similarity is even closer than was supposed from the rough comparison, and that in fact we are dealing here with qualitatively identical expressions of an obviously fundamental biological law.

II

Upon what basis shall any life table function, say l_x, of the *Drosophila* life table be compared with that of man? The life span of one of these organisms is best measured in days, while that of the other is measured in

years. This fact, however, offers no insuperable difficulty to the comparison. What is needed is to superimpose the two curves so that at least two *biologically equivalent* points coincide. The best two points would be the beginning and the end of the life span. But in the case of *Drosophila* our life tables start with the beginning of *imaginal* life only. The larval and pupal durations are omitted. In our preliminary comparison (27) we took human age 15, as the point corresponding biologically to the beginning of imaginal life in *Drosophila.*

I think we can get at this starting point more exactly by putting the human and *Drosophila* l_x curves together as a starting point at the age for each organism *where the instantaneous death rate* q_x *is a minimum.* In the case of *Drosophila,* I think we are safe in concluding, on the basis of the work of Loeb and Northrop (14–17) as well as from our own observations, that this point is at or very near the beginning of imaginal life. We shall accordingly take *Drosophila* age 1 day as this point. Our life tables show that certainly *after* this time q_x never again has so low a value. Indeed the fundamental law of mortality in *Drosophila* imagoes was stated in (27) in this way (p. 492): "the instantaneous death rate increases with age as a modified logarithmic function of x."

The latest edition of Glover's (51) United States Life Tables gives (p. 68) for white males in the original registration states the following values for q_x: for age 11–12 2.28, and for age 12–13, 2.29. We may, therefore, with sufficient accuracy take exactly 12 years as the minimum point, particularly as the l_x figures we shall have to use are tabled as of the *beginning* of the age interval.

For the other end of the life span we may conveniently take the age at which there is left but *one* survivor out of 1,000 starting at age 1 day for *Drosophila* and age 12 years for white males. This age for wild type *Droso-*

phila is, to the nearest whole figure, 97 days. To determine it for white males we have Table I, calculated from Glover's Table 9.

TABLE I

SURVIVORSHIP OF WHITE MALES IN ORIGINAL REGISTRATION STATES ON THE BASIS OF 1,000 AT AGE 12

Age	Number Alive at Beginning of Age Interval l_x	Age	Number Alive at Beginning of Age Interval l_x	Age	Number Alive at Beginning of Age Interval l_x
12–13.....	1,000	45–46	803	78–79	194
13–14.....	998	46–47	792	79–80	171
14–15.....	995	47–48	782	80–81	150
15–16.....	993	48–49	771	81–82	130
16–17.....	990	49–50	760	82–83	110
17–18.....	987	50–51	749	83–84	93
18–19.....	983	51–52	737	84–85	77
19–20.....	979	52–53	725	85–86	63
20–21.....	975	53–54	713	86–87	51
21–22.....	970	54–55	699	87–88	41
22–23.....	965	55–56	686	88–89	32
23–24.....	960	56–57	671	89–90	25
24–25.....	955	57–58	655	90–91	19
25–26.....	950	58–59	639	91–92	14
26–27.....	944	59–60	622	92–93	10
27–28.....	939	60–61	604	93–94	7
28–29.....	934	61–62	585	94–95	5
29–30.....	928	62–63	566	95–96	4
30–31.....	922	63–64	546	96–97	2
31–32.....	916	64–65	525	97–98	1.59
32–33.....	910	65–66	504	98–99	1.01
33–34.....	903	66–67	482	99–100	.63
34–35.....	896	67–68	459		
35–36.....	889	68–69	436		
36–37.....	881	69–70	412		
37–38.....	873	70–71	389		
38–39.....	865	71–72	364		
39–40.....	857	72–73	340		
40–41.....	849	73–74	315		
41–42.....	840	74–75	291		
42–43.....	831	75–76	266		
43–44.....	822	76–77	241		
44–45.....	812	77–78	217		

From this it appears that there is almost exactly one survivor at 98 years. So then we have as biologically equivalent life spans

97 *days* of *Drosophila* life as imago = 86 *years* of human life.

Whence it follows that

1 day of *Drosophila* life = .8866 year of human life
and
1 year of human life = 1.1279 days of *Drosophila* life.

III

We are now in position to make an exact comparison between the life tables of the two organisms. This may be done perhaps most instructively by setting up l_x lines for the two forms on the basis of age in *centiles of the life span,* rather than days or years. That is to say, the whole comparable life spans (as defined in this paper) of 97 days in *Drosophila* and of 86 years for white males will each be divided into 100 equal parts, and the survivors at the attainment of the beginning of each centile interval will then be computed.

This is done for wild-type (long-winged) *Drosophila* males (Pearl and Parker (27) Life Table II) and male whites in original Registration states in 1910 (Glover's Table 9), in Table II.

The two life curves of Table II are shown graphically in Fig. 1, plotted on an arithlog grid. We have, in Table II and Fig. 1, for the first time, so far as I am aware, a precise quantitative comparison of the life spans and one of the mathematical functions of the mortality of two different organisms.

It will be noted that:

1. The form of the l_x distributions is fundamentally the same in both of these organisms over the equivalent life spans. Considering the extreme differences in habits of life, structure, physiology, and environmental stresses and strains in the two cases, this is a truly remarkable result. It seems to me to mean that the factors which determine individual longevity, and differences in this character, are biologically deeply rooted, at least as fundamental, apparently, as the factors which determine the specificity in the morphogenesis of organisms, and perhaps even more so. We are accustomed loosely to think that the prime factors in determining

TABLE II

SURVIVORSHIP DISTRIBUTIONS (l_x) FOR EACH CENTILE OF THE COMPARABLE LIFE SPANS OF (*a*) WILD TYPE DROSOPHILA MALES AND (*b*) WHITE MEN IN THE ORIGINAL REGISTRATION STATES IN 1910

Centile of Comparable Life Span	Numbers Alive at Beginning of Centile Age Interval		Centile of Comparable Life Span	Numbers Alive at Beginning of Centile Age Interval	
	Drosophila	Man		*Drosophila*	Man
0– 1.....	1,000	1,000	51–52	360	673
1– 2.....	991	998	52–53	344	659
2– 3.....	981	996	53–54	328	645
3– 4.....	972	994	54–55	312	631
4– 5.....	963	991	55–56	296	616
5– 6.....	954	989	56–57	280	601
6– 7.....	945	986	57–58	265	585
7– 8.....	935	983	58–59	250	568
8– 9.....	926	980	59–60	235	551
9–10.....	917	976	60–61	221	533
10–11.....	907	972	61–62	207	515
11–12.....	898	968	62–63	193	496
12–13.....	888	963	63–64	180	477
13–14.....	879	959	64–65	167	458
14–15.....	869	955	65–66	155	438
15–16.....	859	950	66–67	143	418
16–17.....	849	946	67–68	132	397
17–18.....	839	941	68–69	121	377
18–19.....	828	936	69–70	111	356
19–20.....	818	932	70–71	102	335
20–21.....	807	927	71–72	92	314
21–22.....	796	922	72–73	84	292
22–23.....	785	916	73–74	76	271
23–24.....	773	911	74–75	68	250
24–25.....	761	905	75–76	61	229
25–26.....	749	899	76–77	55	208
26–27.....	737	893	77–78	49	189
27–28.....	725	887	78–79	43	169
28–29.....	712	880	79–80	38	151
29–30.....	699	874	80–81	34	133
30–31.....	686	867	81–82	30	117
31–32.....	672	860	82–83	26	101
32–33.....	659	852	83–84	22	87
33–34.....	645	845	84–85	19	74
34–35.....	630	838	85–86	17	62
35–36.....	616	830	86–87	14	52
36–37.....	601	822	87–88	12	43
37–38.....	586	814	88–89	10	35
38–39.....	571	806	89–90	9	28
39–40.....	555	797	90–91	7	22
40–41.....	540	788	91–92	6	17
41–42.....	524	779	92–93	5	13
42–43.....	508	770	93–94	4	10
43–44.....	492	760	94–95	3.43	8
44–45.....	475	750	95–96	2.80	6
45–46.....	459	740	96–97	2.27	4
46–47.....	443	730	97–98	1.84	3
47–48.....	426	720	98–99	1.47	2.11
48–49.....	410	709	99–100	1.18	1.37
49–50.....	393	697	100	.94	.87
50–51.....	377	685			

human longevity are such things as the infectious diseases, exposure to unfavorable environment, etc. But *Drosophila,* which so far as is known has no infectious diseases, and in general meets a set of environmental

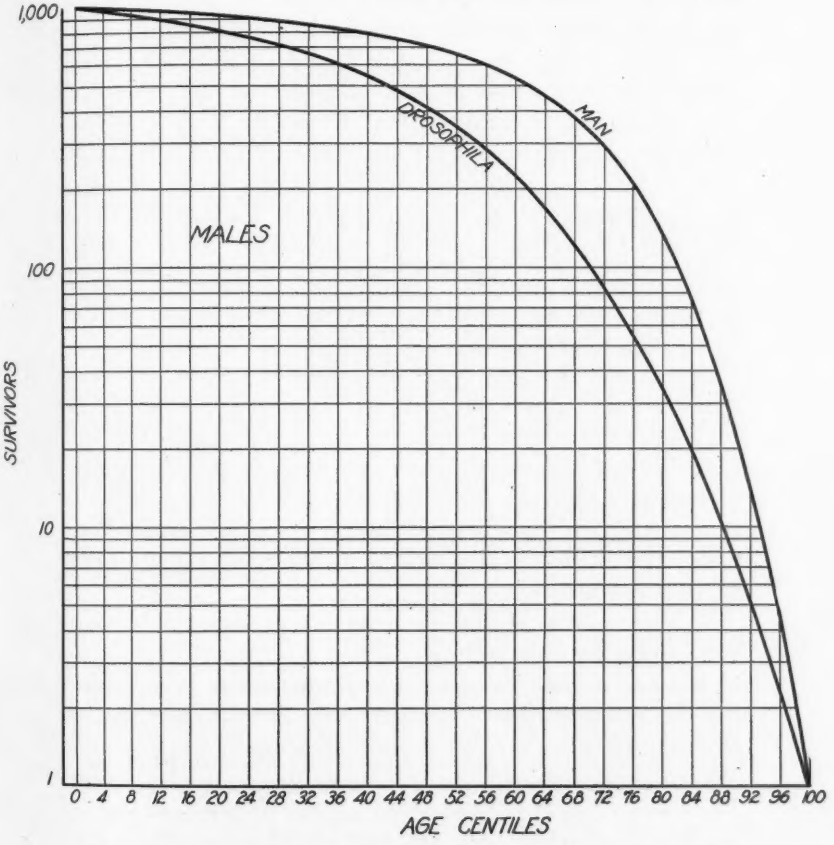

FIG. 1. Comparing the survivorship distributions of *Drosophila* and man (males in both cases) over the equivalent life spans.

conditions wholly different, both qualitatively and quantitatively, from those which operate on man, shows fundamentally the same form of distribution of degrees of longevity.

2. When compared exactly, on the basis of comparable life spans, the human being has at every equivalent age a higher relative expectation of life than does *Drosophila,* measured in terms of its own life span in each case. That this was the case for all but old age was concluded from the rough graphical comparisons of the first Study in this series. It is now seen that the same is true over the whole of life. From this fact the conclusion appears warranted that while the laws of mortality are fundamentally the same *in kind* for *Drosophila* and for man, they differ somewhat quantitatively. There is a temptation to conclude further that the quantitative difference finds its cause in man's own control and amelioration of his environment though sanitation and hygiene. Such a conclusion, however, seems to me not to be strictly warranted, in the light of our present knowledge. There is some suggestion that it is true, as was pointed out in the first of these Studies, from the fact that the progressive change of the human l_x curve in form during historical times has been in the direction of moving from the form typical of *Drosophila* to that now found for progressive, highly civilized groups of men. But definitive conclusions on the point must await further research.

3. The details of the quantitative differences in the two curves are interesting. When the first 25 per cent. of the equivalent life spans has been passed *Drosophila* has lost almost exactly 25 per cent. of the individuals starting life together, while man has lost but 10 per cent. When 50 per cent. of the life spans has been completed *Drosophila* has lost 72 per cent. of the individuals starting together, while man has lost but 31.5 per cent. At 75 per cent. of the life span, *Drosophila* has lost 94 per cent. of the individuals and man 77 per cent. From the 53d centile of the equivalent life spans on practically to the end, man has more than twice as many survivors out of a thousand starting together as does *Drosophila.*

Exactly similar results to those here presented are ob-

tained if one compares human and *Drosophila* life curves for females. Since nothing new in principle is brought out, it is not thought necessary to present the female curves here.

IV

In this paper it is shown that if we take as equivalent life spans in *Drosophila* and man the period between (*a*) the point in the life history of each organism where the specific death-rate (q_x) is a minimum, and (*b*) the point where there is one survivor out of 1,000 starting at the beginning as defined in (*a*), and then divide these equivalent life spans into 100 portions (thus measuring age not in absolute units but in centiles of the life span), the laws of mortality are fundamentally the same in kind in the two organisms. There is a quantitative difference expressible in the statement that at each centile age throughout the life span the number of survivors, out of the same original number starting together, is higher in man than in *Drosophila*.

In a subsequent paper, I hope to take up in detail the functional relations (in a mathematical sense) between the human and *Drosophila* equivalent l_x curves here presented.

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(The plan of numbering citations is explained in the second of these Studies, AMER. NAT., Vol. 56, p. 174.)

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THE SYSTEMATIC LOCATION OF GENES BY MEANS OF CROSSOVER OBSERVATIONS

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ROTHAMSTED EXPERIMENTAL STATION

INTRODUCTORY

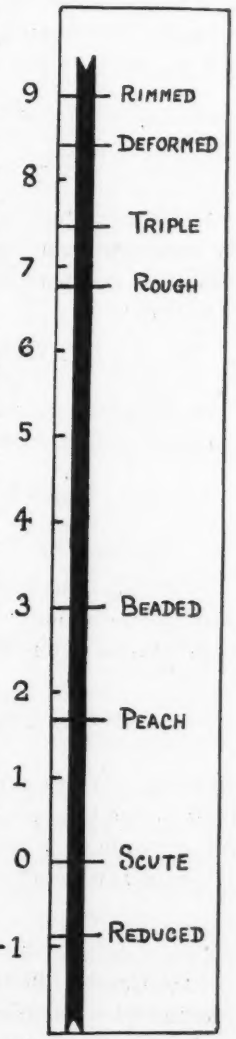
IN the construction of a chromosome map, the distances between neighboring genes are equated to the percentage of crossovers which have been observed between them. Owing to errors of random sampling, and sometimes to other disturbing causes, inconsistencies always arise between the distances so determined. For example, in the important data given by Lancefield and Metz for the sex chromosome of *Drosophila willistoni* [1, p. 241] we have the following values:

TABLE I

	Crossover Percentage	Number of Observations	Number of Crossovers
Scute to Beaded	1.43	279	4
Beaded to Rough	2.42	455	11
Scute to Rough	7.09	6388	453

Within such a small range, double crossing over may be ignored; yet it would be wrong to use such inconsistencies as an argument against the linear arrangement of the genes. For although the true crossover values may be accurately additive, errors of random sampling will certainly disturb the observed percentages. The practical problem is to assign to the distances between the genes values which shall be as far as possible in accord with the whole of the observations available. In other words, we have to make use of as much as practicable, ideally the whole, of the information supplied by the data; giving due weight (i) to the greater accuracy of the values obtained from the larger number of observations, (ii) to the greater accuracy of values obtained from

closer pairs. In general, too, we shall have to consider not three genes only, but a large number, lying sufficiently close together for double crossing over to be ignored, the percentage observed between each pair of which gives indirect information as to the position of all the others.



In its general character the problem resembles those problems involving errors of observation, where a smaller number of unknowns are determined from a larger number of inconsistent equations, and which are usually solved by the method of least squares. The practical solution depends on the construction of a number of "normal equations" for the unknowns, in which the inconsistencies of the data are properly weighted and made to balance. To make the sum of the squares of the errors of the crossover percentages a minimum would, however, be wrong, and the method of least squares is not directly applicable. It has been shown that the whole of the information supplied by the data (2) is made use of by the method of maximum likelihood, and by a first approximation the required normal equations may be constructed.

2. Mathematical Theory

In the above example, if we write p_1 and p_2 for the two adjacent crossover ratios, the probability of the actual series of observations

will be proportional to

$$p_1^4(1-p_1)^{275}p_2^{11}(1-p_2)^{444}(p_1+p_2)^{453}(1-p_1-p_2)^{5935}$$

and the likelihood of any given pair of values for p_1 and p_2 will be proportional to the same quantity. In order to make this quantity a maximum for variations of p_1 and p_2, we have the equations

$$\frac{4}{p_1}-\frac{275}{1-p_1}+\frac{453}{p_1+p_2}-\frac{5935}{1-p_1-p_2}=0,$$
$$\frac{453}{p_1+p_2}-\frac{5935}{1-p_1-p_2}+\frac{11}{p_2}-\frac{444}{1-p_2}=0.$$

These equations are exact, but for practical purposes we need equations linear in p_1 and p_2, and a first approximation is sufficient; if p differs little from $x/(x+y)=x/n$, then

$$\frac{x}{p}-\frac{y}{1-p}=0-\left(\frac{x}{p^2}+\frac{y}{(1-p)^2}\right)\left(p-\frac{x}{n}\right)+\ldots=-\frac{n^3}{xy}p+\frac{n^2}{y}.$$

So that we may rewrite equations (1) in the practical and approximate form

$$\frac{279^3}{4\times 275}p_1+\frac{6388^3}{453\times 5935}(p_1+p_2)=\frac{279^2}{275}+\frac{6388^2}{5935},$$
$$\frac{6388^3}{453\times 5935}(p_1+p_2)+\frac{455^3}{11\times 444}p_2=\frac{6388^2}{5935}+\frac{455^2}{444}.$$

For each percentage observation, therefore, we have merely to calculate the two quantities n^3/xy and n^2/y; then normal equations may be constructed in the form

$$a_{11}p_1+a_{12}p_2+\cdots=b_1$$
$$a_{12}p_1+a_{22}p_2+\cdots=b_2$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$

where a_{12} is the sum of the quantities n^3/xy for which both p_1 and p_2 are involved, a_{11} the corresponding sum for all in which p_1 is involved, and b_1 the sum of the quantities n^2/y for which p_1 is involved.

3. Practical Example

In order to illustrate the practical application of this method to a complex case, we will consider the location of the 8 genes, from Reduced to Rimmed, in the middle

of the sex chromosome of *Drosophila willistoni*. We have here 7 intervals to determine, and fifteen crossover percentages are given [1]. Table II shows the data, and the series of weighting quantities derived from them.

TABLE II

	Percentage	x	n	n^2/y	n^2/xy	Unknowns Involved
Reduced–Scute.....	.95	27	2,848	2,875.26	303,287	p_1
Reduced–Rough....	6.24	37	593	632.46	10,136	p_1, p_2, p_3, p_4
Scute–Peach.....	1.81	8	442	450.15	24,871	p_2
Scute–Beaded.....	1.43	4	279	283.06	19,742	p_2, p_3
Scute–Rough.....	7.09	453	6,388	6,875.58	96,956	p_2, p_3, p_4
Scute–Deformed....	7.24	50	691	744.90	10,295	p_2, p_3, p_4, p_5, p_6
Scute–Rimmed.....	9.91	189	1,908	2,117.78	21,379	$p_2, p_3, p_4, p_5, p_6, p_7$
Peach–Beaded.....	1.70	3	176	179.05	10,504	p_3
Peach–Rough.....	5.05	33	654	688.75	13,650	p_3, p_4
Beaded–Rough.....	2.42	11	455	466.27	19,287	p_4
Rough–Triple.....	.49	4	809	813.02	164,433	p_5
Rough–Deformed ..	2.39	12	503	515.29	21,599	p_5, p_6
Rough-Rimmed....	2.26	62	2,742	2,805.43	124,072	p_5, p_6, p_7
Triple–Rimmed....	1.00	6	601	607.06	60,807	p_6, p_7
Deformed–Rimmed.	4.17	2	48	50.09	1,202	p_7

From this table we write down the normal equations

$$313{,}423p_1 + 10{,}136(p_2 + p_3 + p_4) = 3{,}507.72$$

$$10{,}136p_1 + 183{,}380p_2 + 158{,}509p_3 + 138{,}766p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}103.93$$

$$10{,}136p_1 + 158{,}509p_2 + 182{,}663p_3 + 152{,}416p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}521.58$$

$$10{,}136p_1 + 138{,}766p_2 + 152{,}416p_3 + 171{,}703p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}525.74$$

$$31{,}674\,(p_2 + p_3 + p_4) + 341{,}778p_5 + 177{,}345p_6 + 145{,}451p_7 = 6{,}996.42$$

$$31{,}674\,(p_2 + p_3 + p_4) + 177{,}345p_5 + 238{,}152p_6 + 206{,}258p_7 = 6{,}790.46$$

$$21{,}379\,(p_2 + p_3 + p_4) + 145{,}451p_5 + 206{,}258p_6 + 217{,}460p_7 = 5{,}580.36$$

Using a calculating machine, the work so far is rapid and mechanical; the solution of the normal equations may in this case be much simplified by observing the uniformity of some of the sets of coefficients, a type of uniformity which is probably characteristic of crossover data. Thus by considering $(p_2 + p_3 + p_4)$ as a single quantity, p_1 is immediately expressible in terms of it, and by solving the last three equations we may do the same for p_5, p_6 and p_7; substituting finally in equations (2, 3,

4) we solve them for p_2, p_3 and p_4, and obtain the values shown in Table III.

The seven values obtained give mutually consistent values for the crossover percentages between the fifteen pairs tested, and are therefore suitable for the construction of chromosome map. If the conditions of Maximum Likelihood had been exactly fulfilled they would agree better than any other consistent series of values with the percentages observed. As it is, it is only in the aberrant value of p_7 that the assumption that the observed values are approximately correct breaks down, and it is probable that such cases will only occur when the data are admittedly insufficient.

TABLE III

	Calculated	Observed	Difference d	Standard Error σ	$\frac{d^2}{\sigma^2}$
Reduced–Scute	.90 p_1	.95	+ .05	.18	.08
Reduced–Rough	7.66	6.24	−1.42	1.09	1.70
Scute–Peach	1.67 p_2	1.81	+ .14	.61	.05
Scute–Beaded	2.98	1.43	−1.53	1.02	2.31
Scute–Rough	6.76	7.09	+ .33	.31	1.13
Scute–Deformed	8.40	7.24	−1.16	1.06	1.20
Scute–Rimmed	8.97	9.91	+ .94	.65	2.09
Peach–Beaded	1.31 p_3	1.70	− .39	.86	.21
Peach–Rough	5.09	5.05	− .04	.86	.00
Beaded–Rough	3.78 p_4	2.42	−1.36	.89	2.34
Rough–Triple	.69 p_5	.49	− .20	.29	.48
Rough-Deformed	1.64	2.39	+ .75	.57	1.73
Rough–Rimmed	2.21	2.26	− .05	.28	.03
Triple–Rimmed	1.52	1.00	− .52	.50	1.08
Deformed–Rimmed	.57 p_7	4.17	+3.60	1.09	10.91
				$\chi^2 = 25.34$	

Table III is arranged to compare the differences between the calculated and the observed percentages with the standard errors due to sampling; except for p_7 all the differences are less than twice their standard errors; thus showing the general agreement between the data and the theory of linear arrangement of the genes. The fit, however, is not a close one, even if we omit p_7; in the present state of our knowledge this will not throw any

doubt on the scheme of linear arrangement, but will suggest that the crossover ratios in this part of the chromosome were not constant in all the strains used to compile the data.

In estimating the Goodness of Fit of data of this kind, χ^2 may be calculated by summing the values of d^2/σ^2, as in Table III Attention should, however, be called to the fact that it has been recently shown (3) that in entering Elderton's Table we must put n' equal to one more than the number of degrees of freedom, remaining after we have fitted our unknowns to the data. In the present case we have found 7 unknowns from 15 equations, leaving 8 degrees of freedom, so that n' should be 9, and not 16.

In conclusion it should be noted that to be available for the use of this process the crossover data should be stated in the form in which it is given by Lancefield and Metz, in which the crossovers tabled between any two genes do not include those experiments in which an intermediate gene was under observation. The practice of throwing together all the crossovers between two genes, in order to improve the ratios between the more distant points, causes the same crossover to appear repeatedly in different entries. The data are no longer the product of independent experiments, and must be re-summarized before reduction.

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LINKAGE IN PEROMYSCUS

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STUDENTS of Mendelism are beginning to display the same interest in possible homologies between the genetic factors or "genes" of different species of animals or plants which the morphologists of thirty years or more ago did in homologies between organs. In considering a given case of suspected homology between genes, two criteria are, so far as I know, employed: (1) Resemblance between the developed characters which are attributed to the action of supposedly homologous genes. Mere similarity of appearance, however, is recognized as an extremely fallible criterion of homology here as in the case of comparative anatomy. (2) Agreement between the "cross-over" value shown by a pair of linked factors in one species, as compared with the corresponding value shown by supposedly homologous factors in another species. If both of the two linked genes under consideration are found to have much the same somatic effects in the two species, and if, furthermore, the degree of linkage is approximately the same in the two cases, the argument is strong for a twofold homology.

Metz[1] and Sturtevant[2] have been investigating the parallel mutations of several species of *Drosophila,* and it is not unlikely that this genus will furnish the best material for the study of genic homologies, just as it has shown incomparable superiority for certain other lines of genetic research.

For rodents, what appear to be parallel mutations have been shown to occur among numerous species, even ones

[1] *Genetics,* March, 1918.

[2] *Genetics,* January, 1921.

belonging to widely different families.[3] In one case, that of the mutation known as "pink-eye," not only is the visible modification closely similar in rats and mice, but the linkage relations between this factor and that for albinism are known to be of the same order of magnitude in the two animals.[4]

Some years ago, Castle[5] described two similar mutations in the Norway rat, which he termed "pink-eyed yellow" and "red-eyed yellow," respectively. These, according to the published descriptions, differ chiefly in the color of the eyes, the latter variety having darker eyes than the former. These two mutations, and likewise true albinism, were all found to result from the modification of distinct genetic factors. Any two of them, when crossed, gave rise to the wild type in the first hybrid generation. On the other hand, further breeding tests led Castle to conclude that all three of these factors were linked. When red-eyed and pink-eyed rats were interbred, the cross-over percentage proved to be about 18. When pink-eyed rats were crossed with albinos, this value proved to be about 21. On the other hand, the linkage between red-eye and albino proved to be almost absolute. One hundred and sixty F_2 albinos and 57 F_2 red-eyed yellows, when mated with pure red-eyes and albinos, respectively, yielded but a single offspring which was not of the wild type.

More recently Dunn[6] has tested the linkage between this same red-eyed condition and albinism in the rat. From his own data he computes a cross-over value of 1.8 per cent., but when his data are combined with those of Castle, this value falls to less than one per cent.

Castle and Dunn have likewise tested the degree of linkage between "pink-eye" and albinism in the mouse

[3] Dunn has compiled these cases in a useful article in the *Journal of Mammalogy*, August, 1921.

[4] According to Castle, the percentage of cross-overs is 21 for rats and 14 for mice. This may or may not be construed as evidence of "homology."

[5] AMERICAN NATURALIST, February, 1914; *Science*, August 6, 1916 (with Wright); Carnegie Institution Publications 241 and 288.

[6] *Genetics*, May, 1920.

(*Mus musculus*). The proportion of cross-overs was found to be about 14 per cent.

Some five years ago I described a pale, red-eyed mutant of *Peromyscus*,[7] which originated among the offspring of three sibs in the F_2 generation of a cross between *P. maniculatus rubidus* and *P. m. sonoriensis*. Since I have already described this "mutant" race rather fully, and since it will again be discussed shortly in a paper by Mr. H. H. Collins and myself, I need not enter into a detailed account of it here. I have not seen specimens of the "red-eyed yellow" rats described by Castle, but I find little in the description of that race which is at all at variance with my own "pallid" race of *Peromyscus*. The latter has undergone a great reduction of the black pigment, while the yellow pigment has been little if any affected. The eyes are commonly dark red, rather than pink, though they present a considerable degree of variability, ranging from a condition not much darker than the true pink of albinos to a condition not much paler than the normal. There are, however, no real intergrades between the pallid mice and the wild type, and the behavior of this complex of characters in crosses is that of a simple monohybrid recessive. Furthermore, it is not an allelomorph of albinism, since the wild type alone results from matings between albinos and pallids.

I have recently carried out tests of the linkage relations between this factor and that for albinism.[8] Thus far, it has not been found practicable to devote any considerable proportion of my time to this phase of the subject, and the numbers are accordingly inadequate for any exact measurement of cross-over values. They are, none the less, sufficient to show the existence of a high degree of linkage between these factors. The number

[7] *Genetics*, May, 1917; AMERICAN NATURALIST, August–September, 1918. This mutant was at first referred to as a "partial albino"; later the noncommittal term "pallid" was applied to it.

[8] The albinos used were all derived from a single brood belonging to the subspecies *Peromyscus maniculatus gambeli*.

of F_2 individuals derived from simple F_1 x F_1 matings is too small to give a representative dihybrid ratio. The really important tests have been made with "extracted" albinos and pallids of the F_2 generation.

Matings have been made (1) between " extracted " albinos and "pure" pallids (*i.e.*, those known to be free from the factor for albinism), (2) between extracted pallids and pure albinos, and (3) between extracted pallids and extracted albinos. There were likewise a number of matings in which the pedigrees were less simple.[9] On the assumption of a wholly independent segregation of these factors, our F_2 pallids (of simple pedigree) should have a 2/3 chance of being heterozygous for albinism, while our F_2 albinos should have a 3/4 chance of being either homozygous or heterozygous for pallid.[10]

Eighteen F_2 mice were involved in these tests. The total number of offspring derived from these was 135, the number per parent ranging from 3 to 26. By no means all of these parents, taken singly, have thus far given birth to a sufficient number of young to prove their genetic composition with any certainty. But the cumulative testimony of all of these matings is overwhelming. Not a single pallid mouse and only two albinos have appeared among the 135 young which have thus far been born. Had there been a normal proportion of "carriers" among the parents, these matings should have yielded 37 albinos and 18 pallids, as the most probable "expected" numbers. That all of the offspring with two exceptions (these being sibs) were of the wild type is evidence of a high degree of linkage (in this case "repulsion") between the albino and the pallid factors.[11]

[9] Back-crosses and heterozygous albinos figured in some of these pedigrees. In these cases the odds are different from those which hold for individuals derived from the simpler types of mating. They have, however, been computed for every animal used. In about half of the "extracted" albinos, for example, there was only a 5/8 chance that the individual carried the pallid factor.

[10] It is a safe assumption that the double recessive form would be albino.

[11] It might be supposed that the testimony of 18 parent mice, even if all of these were shown conclusively to be lacking in "cross-over" gametes,

From these considerations we may regard it as not unlikely that my "pallid" race of *Peromyscus* has resulted from the mutation of a genetic factor homologous with that which has mutated in the case of Castle's "red-eyed yellow" rats.

This decisive result, as regards the existence of linkage between the pallid and albino factors in *Peromyscus*, stands in contrast with the apparent absence of such linkage in another cross between mutant strains of these mice. Albinos were mated with mice belonging to a strain which I have elsewhere referred to rather inappropriately as "yellows."[12] The latter vary from clay color to a distinctly reddish hue, according to the strain, and are characterized primarily by a marked increase in the length of the "agouti" cross-band and by a decrease in the proportionate number of all-black (unbanded) hairs in the pelage. Where present, however, the black pigment is of full intensity. This applies to the basal zone of the body hairs, both dorsal and ventral, to the black hairs of the dorsal tail stripe, as well as to the eyes, ears and soles of the feet.

Matings between albinos and "yellows" have resulted exclusively in F_1 mice of the wild type (dark). An F_2 generation of 83 was obtained, consisting of 52 dark individuals, 13 yellows and 18 albinos. On the assumption of purely random assortment of gametes, the "expected" numbers are 44, 15 and 20, respectively. The observed numbers are doubtless within the range of "accidental" variability. In any case they give no evidence

would not be sufficient to prove the existence of linkage. It should be repeated, however, that we are not here dealing with cases in which there would be merely an equal chance of combining the two mutant factors in the same individual. The odds in favor of this (linkage aside) may, as stated above, be as high as 2 to 1, or even 3 to 1. Thus, the likelihood of obtaining, by chance alone, 17 non-cross-over cases out of 18 becomes vanishingly small.

[12] *Genetics*, May, 1917; AMERICAN NATURALIST, August–September, 1918. A more complete account of these mice, dealing with two subvarieties differing somewhat in color, is included in a forthcoming paper by Mr. H. H. Collins and myself.

of linkage, the occurrence of which would have reduced the proportionate number of dark individuals, instead of increasing it.

The number of F_2 albinos and yellows which have been thus far tested is very small, but it is of interest that the proportion of recombinations is even greater than would be expected from random assortment. Inclusion of these meager results in the present report seems justified by the probability that we shall not soon rear any considerable number of hybrids between the yellow and albino varieties.

Seven extracted albinos have been mated with pure yellows. Three of these have given only yellow offspring, the numbers being 9, 13 and 21, respectively. Thus, three of these seven albinos are, in all probability, double recessives (*ccyy*). (One in four should be double recessives, according to chance.) Three other albinos have given mixed offspring. They are evidently of the formula *ccYy*. The remaining one appears to have the formula *ccYY*, as judged by the production of 15 dark young.

Two extracted yellow females mated with a (supposedly) pure albino male gave birth to 4 albinos and 4 dark.[13] No albinos would be expected here if linkage were complete, while only one third should be albinos in the total absence of linkage. Thus the number of recombinations is again too high, even on the assumption of no linkage.

These numbers are, of course, very small. But even here such proportions would have been quite improbable had any marked degree of linkage existed—such, for example, as has been found to exist between the pallid and albino factors.

[13] It is only fair to add that 4 yellows likewise resulted from these matings. This was doubtless due to the fact, unsuspected at the time, that the albino male carried the "yellow" factor, one of his *two* great-grandparents having been heterozygous for yellow.

THE SOUND-TRANSMITTING APPARATUS OF SALAMANDERS AND THE PHYLOGENY OF THE CAUDATA

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RESEARCHES by Kingsbury and Reed, extending through a number of years, have shown that the sound-transmitting apparatus of salamanders consists of two elements. These are the columella and the operculum.

In the most recent paper on this subject, Reed (1920) gives a résumé of all the previous work, an extensive account of the state of affairs in the *Plethodontidæ,* a brief account of the conditions in other forms, and the findings are presented in the form of a family tree.

The purpose of the present article is to add an account of the condition of the apparatus in two forms not seen by Reed, to question the condition described by Kingsbury and Reed for *Dicamptodon ensatus* (*Ambystoma tenebrosum* Auct.), to suggest a somewhat different interpretation of the facts observed by them, and to propose a somewhat different phylogeny, which seems to agree quite as well with the otic apparatus and far better with other anatomical features.

Kingsbury and Reed (1909) were unable to examine any of the Asiatic forms related to *Hynobius.* These forms, as Cope pointed out long ago, are rather different from the *Ambystomidæ,* with which they have usually been associated, and should in fact form a family *Hynobiidæ.*

I have recently been able to examine large series of *Hynobius leechii* from Korea. This animal shows a condition of the otic apparatus different from any seen by Kingsbury and Reed, and a condition which I am compelled to consider primitive. Both columella and oper-

culum are present as free and distinct elements. Both are readily movable. There is a m. opercularis.

I have not been able to examine skulls of *Onychodactylus,* or of *Ranodon.* Okajima's (1908) figures of *Onychodactylus* show only one element which is in appearance much like that of *Cryptobranchus.* This is very different from the appearance of the apparatus of *Hynobius.* It is evident that either fusion of operculum and columella has taken place or that the operculum has not developed. *Onychodactylus* is partly aquatic, a mountain brook animal. *Cryptobranchus,* which, as I shall show later, is a derivative of the *Hynobiidæ,* has failed to develop the operculum. Probably the same is true of *Onychodactylus* and of *Ranodon* as well, although for the latter Wiedersheim's (1877) figure is all we have. Still, as Kingsbury and Reed (1909) say, his Fig. 67 "suggests a condition such as is found in *Cryptobranchus.*"

Rhyacotriton olympicus was not examined by Kingsbury or by Reed. This animal (Dunn, 1920) possesses both columella and operculum. The columella is free from the periotic and is readily movable. The operculum is little developed. The animal is in part aquatic, a mountain brook species.

Dicamptodon ensatus was examined by Kingsbury and Reed (1909), and while my dissection of an adult showed the state of affairs which they describe, I can not follow them in calling it "much like that in the adult *Ambystoma.*" In adult *Ambystoma* the columella is solidly fused to the periotic. A bony operculum nearly fills the opening of the fenestra, and is attached by a membrane around its circumference. In *Dicamptodon,* on the other hand, about half of the fenestra is filled by the plate of the columella, and the remainder by cartilage. The cartilage extends around the plate of the columella. There is nothing that could be called a definite operculum. If the cartilage is called the operculum, then the columella and operculum are fused and the operculum is fused to

the ear capsule by nearly its whole border. It seems to me that in this case the columella is at least more free than in *Ambystoma,* and the operculum less developed. This would be in line with what is known of the habits of *Dicamptodon.* It is a much more aquatic animal than is *Ambystoma.*

In the Caudate sound-transmitting apparatus, taking Reed (1920) as a basis, there are the following sets of conditions:

I. Both columella and operculum present. Both free.
Hynobius, Rhyacotriton.

II. Operculum not developed. Columella free.
Cryptobranchus.
Megalobatrachus Ranodon ?,
Onychodactylus ?, Dicamptodon ?

III. Operculum developed, free. Columella fused to periotic, stylus present.
Salamandra, Ambystoma.

IV. Operculum developed, free. Columella fused to periotic. Stylus absent.
Triturus, Pachytriton, Pleurodeles ?,
Tylototriton ?.

V. Operculum developed, free. Columella ?.
Siren, Batrachoseps.

VI. Both columella and operculum present, fused together, free from periotic.
Necturus.

VII. Both columella and operculum present. Fused together. Operculum attached by narrow fusion to periotic.
Amphiuma, Plethodontidæ
(exc. *Batrachoseps*).

Inasmuch as II is a condition found also in larvæ, there is no reason to suppose that the animals in which this condition occurs form a natural group.

Condition V has been commented upon by Reed (1920), and I am fully in accord with his ideas in this connection. *Siren* and *Batrachoseps* are certainly not related. Both are extremely specialized. *Batrachoseps* has certainly passed through stage VII. *Siren* has certainly passed through an ancestral period of terrestrial life, yet its other peculiarities are such that it is dangerous to state that its relationships are with the forms in stage IV.

The forms which show condition VI and condition VII

form what Reed (1920) calls Legion II, as distinct from the forms which show conditions I–V (exc. *Batrachoseps*), which Reed calls Legion I.

But the sound-transmitting apparatus of *Necturus* agrees with that of *Amphiuma* and the *Plethodontidæ* only in having the columella and operculum fused. There is no reason to suppose that such a fusion may not have occurred twice, especially as the details of the fusion in *Necturus* differ somewhat from the manner in which the fusion occurs in *Amphiuma* and the *Plethodontidæ*. In *Necturus* the columella forms a goodly part of the plate-like portion of the apparatus. In the forms of condition VII, the plate-like portion is almost entirely composed of the operculum, and the columella is represented by the stylus. In this case the evidence of the ear bones is non-committal. Considered apart from all other features of the anatomy condition VII might equally well be derived from condition VI or both independently from condition I. But, as we shall see, evidence from other features of the anatomy precludes our regarding *Necturus* as intervening between the *Plethodontidæ* and the other Mutabilian forms.

It is extremely interesting to note that Reed has found almost exactly the same state of affairs in *Amphiuma* and in the *Plethodontidæ*. The exact relationships of *Amphiuma* have long been in dispute, and while I prefer to be conservative about the position of the animal, I think it extremely likely that further evidence will show that it is closer to the *Plethodontidæ* than it was placed in the older classifications.

Any classification should be based upon all available characters, so that possible parallelisms will not lead to wrong conclusions. In the present instance we are dealing with a stock neither absolutely terrestrial nor absolutely aquatic. From this stock there have been several branches which have become more aquatic and several which have become more terrestrial. Excellent examples of this are the numerous incursions into a mountain

brook habitat, with the penalty of loss or reduction of lungs. The list is extensive, *Onychodactylus, Rhyacotriton,* four species of *Triturus, Salamandrina, Chioglossa,* all the stock of the *Plethodontidæ,* an assemblage representing four families. The sound-transmitting apparatus is admittedly correlated with the mode of life. Therefore as a character in determining relationships it must be used with extreme caution.

The following outline classification of salamanders does not counter any of the facts concerning the otic apparatus, and is based on many characters.

As regards the *Plethodontidæ* and the *Hynobiidæ,* revisions of both are nearly completed, based on the examination of some 8,000 specimens of the first family and 1,000 of the second.

The *Sirenidæ* are the most isolated group. Scarcely a character can be found to ally them with one or another of the main stocks. The pelvis is gone, the skull is that of a very specialized larva, the hyoids are those of almost any larva, the tail vertebræ are very different from those of any other salamander, inasmuch as there is no hæmal arch. There are flat plates on each side which do not meet in the mid-ventral line. There is no prearticular.

The *Proteidæ* are only slightly less isolated. The pelvis differs in having an anterior median projection and no ypsiloid apparatus. The skull is larval. The branchial arches are reduced from the primitive larval quota. The prearticular is absent.

The *Amphiumidæ* also have modified larval branchial arches, and the pelvic girdle lacks the ypsiloid apparatus. But *Amphiuma* has an adult skull which resembles remotely that of the *Salamandridæ.* The otic apparatus is that of the *Plethodontidæ.* There is no prearticular bone. It is quite possible that this genus is descended from primitive Salamandrids.

The others have directly comparable skulls, branchial arches, and pelves, and in dealing with their relationships we are on much firmer ground.

Several characters divide them into two series, which should, I think, rank as superfamilies.

1. Prearticular bone. Present in *Cryptobranchidæ* and *Hynobiidæ*, and absent in *Ambystomidæ*, *Salamandridæ* and *Plethodontidæ*.
2. Second epibranchial. Present in *Cryptobranchidæ* and *Hynobiidæ*, and absent in *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ*.
3. First ceratobranchial and first epibranchial fused into a single cartilaginous rod in *Cryptobranchidæ* and in *Hynobiidæ*. Separate elements in *Ambystomidæ*, *Salamandridæ* (exc. *Salamandra*, where all parts fuse), and *Plethodontidæ*.
4. Nasals meeting in median line and premaxillæ without nasal process in *Cryptobranchidæ* and *Hynobiidæ*. Nasals separated by nasal spines of premaxillæ in *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ* (exc. *Pseudotriton*, where nasals overlap premaxillary spines).
5. Pubotibialis muscle fused with puboischiotibialis in *Cryptobranchidæ*. The two muscles are separate in all other salamanders (Noble, 1922). I have ascertained that the two are fused in *Hynobius* and in *Onychodactylus*.
6. Larvæ of *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ* have the first ceratobranchials fused with the second basibranchial (Smith, 1920). This fusion does not occur in larvæ of *Cryptobranchidæ* or of *Hynobiidæ*.

Within the superfamily *Salamandroidea* the *Ambystomidæ* and the *Salamandridæ* are about parallel. The long posterior process of the prevomer distinguishes the *Salamandridæ*, and as the parasphenoid tooth patches of *Plethodontidæ* are the morphological equivalent of this process (Wilder, 1920) it is probable that some primitive Salamandrid (having the two otic elements free) gave rise to the much degenerate *Plethodontidæ*. The

mountain brook habitat of the ancestral Plethodontid (Wilder and Dunn, 1920) accounts perfectly for the retention of the columella through adult life as a working part of the sound-transmitting apparatus.

The *Cryptobranchoidea* contains two families. Of these the *Hynobiidæ* is the more primitive. The *Cryptobranchidæ* differ in lacking the lachrymal bone, in the larval position of the vomerine teeth, and in the much depressed form of the body and head, the last two evidently adaptations for aquatic and bottom-living habits. Besides the characters mentioned in the list as aligning the *Cryptobranchidæ* with the *Hynobiidæ*, several minor points also show this relationship. Both *Ranodon* and *Hynobius* frequently have a lateral fold between the insertions of the legs. This is very prominent in both *Cryptobranchus* and in *Megalobatrachus*, and is not found elsewhere. *Onychodactylus* larvæ have a marked fold on the posterior side of the limbs. This is seen elsewhere only in *Cryptobranchus* and in *Megalobatrachus*.

Inasmuch as the characters differentiating the two genera of *Cryptobranchidæ* have not been clearly understood in the past they are here stated.

Megalobatrachus, Two persistent branchial arches:
Frontal not entering naris:
Branchial clefts closed in adult.

Cryptobranchus, Three persistent branchial arches:
Frontal entering naris:
Branchial clefts open in adult.

In all three of these characters the American genus shows greater adaptation to aquatic life. The European fossils of this family appeal to *Megalobatrachus* in the one skull character which separates the two genera. Neither in *Andrias schuchzeri* nor in *A. tschudii* does the frontal enter the naris.

It is also interesting to note that *Megalobatrachus* shows no "Derotreme" characters whatever, although in the older classifications it was included in the *Derotremata*.

The extreme antiquity of the *Caudata* can be readily seen when an end form, a river adaptation, is found in Oligocene times.

This of course puts the origin of the main stocks back at least to the end of the Mesozoic, a conclusion to which the distribution also forces us.

The primitive characters appear in widely scattered and rather unrelated forms. The free prearticular has already been mentioned. A free lachrymal is found in *Hynobiidæ* and in an Ambystomid, *Rhyacotriton*. A postfronto-squamosal arch is found in one group of the *Salamandridæ*. A T-shaped parasphenoid is found in an Ambystomid (*Dicamptodon*) and in a Salamandrid (*Tylototriton*). Long maxillæ are found in the two forms just mentioned and in another Salamandrid, *Pachytriton*. Posteriorly projecting prevomers are found in *Amphiuma*, in all *Salamandridæ*, in some *Hynobiidæ* (*Hynobius, Pachypalaminus*), and to a less extent in *Dicamptodon*.

All these are theoretically primitive skull characters of amphibians. Their appearance separately in diverse forms is sufficient indication that the three families *Hynobiidæ, Salamandridæ,* and *Ambystomidæ*, while containing all the more primitive forms of the order, stand in no direct genetic relationship to each other, but must be derived from a more or less remote common stock which combined the otic apparatus, lachrymal, and prearticular of *Hynobius* with the long maxilla, T-shaped parasphenoid, and postfronto-squamosal arch of *Tylototriton*.

The evidence of Paleontology, as far as it goes, supports this view. I intend in a later paper to assemble the meager facts regarding fossil salamanders. These facts, it may be here stated, lend no support to the prevalent view that the *Proteida* are an old, a primitive, or an ancestral group.

The following outline classification indicates the size and position of the modern groups. The genera and

species of the *Salamandridæ* are probably not wholly accurate. Future work will perhaps indicate the affinities of *Amphiuma*, the *Proteidæ*, and the *Sirenidæ*.

Of the larger families, the *Hynobiidæ* are entirely Asiatic, the *Salamandridæ* are Eurasiatic with four American species, the *Plethodontidæ* are American with two species in Europe and four in South America, and the *Ambystomidæ* are American with one Asiatic species. As the Northern land masses have been connected with each other during Tertiary times this distribution is not extraordinary, although close resemblance between widely separated species is eloquent testimony as to the antiquity of some of the "modern" forms.

Twenty-two of the recognized genera and 105 of the species are restricted to North America, 13 genera and 56 species are Eurasiatic, while three genera are found both in North America and in some parts of the Old World.

Mutabilia

Salamandroidea

1. Ambystomidæ .....3 genera, 16 species.
   Dicamptodon 2, *Rhyacotriton* 1, *Ambystoma* 13.
2. Salamandridæ .....7 genera, 37 species.
   Salamandra 5, *Chioglossa* 1, *Tylototriton* 2, *Pachytriton* 1, *Pleurodeles* 3, *Triturus* 24, *Salamandrina* 1.
3. Plethodontidæ .....16 genera, 83 species.
   Desmognathus 7, *Leurognathus* 1, *Plethodon* 11, *Ensatina* 3, *Hemidactylium* 1, *Aneides* 4, *Batrachoseps* 6, *Stereochilus* 1, *Typhlotriton* 1, *Typhlomolge* 1, *Gyrinophilus* 2, *Pseudotriton* 5, *Eurycea* 10, *Hydromantes* 3, *Œdipus* 23, *Œdipina* 4.

Amphiumoidea (Relationships uncertain, possibly should stand as a family under Salamandroidea)

4. Amphiumidæ .....1 genus, 2 species.
   Amphiuma 2.

Cryptobranchoidea

5. Hynobiidæ .....5 genera, 20 species.
Hynobius 15, *Pachypalaminus* 1, *Onychodactylus* 2, *Ranodon* 1, *Batrachuperus* 1.

6. Cryptobranchidæ .....2 genera, 2 species.
Megalobatrachus 1, *Cryptobranchus* 1.

Proteida (Relations uncertain)

7. Proteidæ .....2 genera, 3 species.
Necturus 2, *Proteus* 1.

Meantes (Relationships uncertain)

8. Sirenidæ .....2 genera, 2 species.
Siren 1, *Pseudobranchus* 1.

Total number of genera 38, of species 165.

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AGENCIES WHICH GOVERN THE DISTRIBUTION OF LIFE

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THE problems presented by the distribution of plants and animals is a fertile field for investigation. These problems are essentially ecological in character, for often, perhaps always, the range of a species or genus is dependent upon a number of diverse environmental factors, some of which are readily apparent, while others are obscure; but always they merit careful study.

In investigating and mapping the ranges of living organisms and in following the evolutional tendencies of species in so far as we are able, environment and its influences are of the greatest moment, especially from an ecological standpoint. Botanical subjects may usually be allocated in relation to their surroundings with considerably greater ease than can active forms of life, for the former are acted upon only by the agencies to be found in one spot, while the latter may experience not only all the influences operative over several square miles, more or less, of diversified territory, but, in the case of a migratory bird or mammal, will be subject during a part of the year to environmental factors of which we may know nothing. Whether a species is common or rare in a certain area depends upon its rate of reproduction, which is usually entirely adequate unless new and disturbing influences have been introduced; upon the number of favorable or unfavorable conditions which it encounters, the amount of competition with which it has to contend, and its phylogenetic characters, as to whether it be of a plastic type or one which is senescent and overspecialized: all of which may be summed up in the phrase "adaptability to its habitat."

In any one realm, or larger region of the earth's surface, there are various climatic divisions, the chief of which have been named zones, and these stretch across the continent following isotherms, or mean temperature bands, usually, for our purpose, based upon the average amount of heat present during the three chief reproductive months. Zones are divisible into faunal districts, whose bounds are limited by conditions of humidity, precipitation and a few other causes that may be operative over considerable areas. These are further divisible into associations, an almost limitless number of which may be recognized. Thus, we have littoral, riparian or stream bank, palustral or marshy associations, the latter being capable of still narrower subdivision into tule, arrow-head or salt grass associations, and so on without end.

Associations are sometimes but little considered in parts of the country where climatic conditions are uniform over a wide extent of territory; but in the mountainous parts of the west, where practically every possible local environment from the hottest, most arid deserts, to arctic-alpine conditions may be encountered within a few miles, the importance of their recognition can hardly be overestimated.

In considering the agencies governing the range of a form, the question of temperature is undoubtedly of chief importance as a usual thing, but in some cases physical barriers should be given greater weight, for it need hardly be indicated that it is such directly—and temperature only indirectly if at all—that keep many forms of life from greatly increasing their ranges. In studying such barriers, manner of dispersal may be of much importance. In the case of plants more than of vertebrates (with few exceptions), human agency must now be taken into account, for the activities of man, both intentional and unintentional, are responsible in greater degree for the widespread dissemination of seeds and insects over vast stretches of the earth's surface than

any other cause. Natural manner of dispersal must also be carefully scrutinized as a preliminary step, for what will prove a barrier to the extension of the range of a plant with what I may term unadorned seeds may be inoperative in the case of seeds adapted to dispersal by the wind, and again, those whose covering is fitted for adhesion to the coats of mammals will often be still more widely scattered.

In the case of an animal, the first thing to be considered is the life-type to which it belongs, the chief divisions of which are aquatic, fossorial, terrestrial, arboreal and volant types, which are limited in varying degrees by physical barriers. To an aquatic form, land masses are insuperable obstacles, while to many terrestrial species, especially such as live in very arid regions and are totally independent of water, even a large river may prove a delimiting agent. A strip of rocky country or an extent of arid plain will prevent the spread of such a fossorial mammal as the mole. Arboreal forms are checked by large, treeless areas, and animals which are adapted to a life on the plains will usually shun the forests. Volant types are the most independent of physical barriers of all, and to some even wide stretches of ocean are no obstacle, as in the case of the Pacific Golden Plover (*Charadrius dominicus fulvus*), in its annual migrations between Alaska and the Hawaiian Islands.

While conceding that temperature is the most important factor in the distribution of life, the writer is of the opinion that not enough importance has been credited to other agencies. Dr. C. H. Merriam was, I believe, the first to formulate the theory that the northward range of a species is governed by the mean amount of heat present during the season of reproduction, while the southward range of northern forms is restricted by the mean temperature during the very hottest portion of the year. Isotherms have been determined and our continent plotted and mapped into zones, called Arctic, Hudsonian, Canadian, Transition, Upper Sonoran, Lower

Sonoran and Tropical, some of which are known by other terms in the eastern part of the country. Roughly, the position of an isotherm, as well as the temperature of a region at other times of the year, depends upon latitude, altitude and distance from the sea. Hence it is that the winter temperature of parts of Montana at a considerable altitude and far from the sea reaches a lower figure than has been recorded on the coasts of the Arctic Ocean. The coldest temperature ever known upon the face of the earth—minus 92 degrees F. in the interior of Siberia—is much lower than has ever been found by any of the "farthest north" expeditions.

We may safely infer that the degree of winter cold, below a certain point, is largely immaterial, for it makes no difference to a tree whether the thermometer is ten or sixty degrees below zero, nor to the lesser vegetation and many rodents safely protected by a deep blanket of snow. Even to the few species of birds which habitually spend the winter in high latitudes, very low temperatures are seldom disastrous, but rather is it due, when numbers perish, to a failure of the food supply during sleet storms or long blizzards. Neither birds nor mammals migrate so much because of cold as because their usual foods are not to be obtained in adequate amounts during the winter.

Certain forms of life may have to contend, in relatively low latitudes and altitudes, with conditions which approximate those to be found much farther north. W. T. Shaw has but just brought to our attention the fact that in eastern Washington, where Upper Sonoran conditions are the rule, estivation and hibernation of the Townsend Ground Squirrel (*Citellus townsendi*) are so long continued that this animal enjoys but four months of activity during the year. The squirrels emerge as soon as the first growth starts in the spring, but retire to their burrows for the long sleep when the arid conditions of early summer cause a desiccation of their food supply. To a torpid animal in its nest below ground it

makes no difference whether it is summer or winter above, and so these squirrels seem to lead an existence closely similar to that of their near kin at the Arctic Circle, but with the probable difference that the northern forms experience an actually greater number of hours of daylight throughout the long arctic summer months.

In the plains section of the interior, zonal divisions are acted upon by comparatively few modifying agencies, and their boundaries are rather regular and easily defined, but in parts of the three Pacific Coast states, whose shores are bathed by warm ocean currents, and where the topography is decidedly irregular, the problem of zonal definition is often extremely complicated. In the coast region of northern California, for instance, there is but slight daily and seasonal change of temperature, and a number of Boreal forms are able to occur there because the summers are cool enough for them, while certain Sonoran species are also able to exist because the mean temperature of the breeding season is high enough for their needs. The result is a confusion of zonal indices that is extremely puzzling at first glance.

To these three widely-recognized zonal factors, when operative in certain regions, should undoubtedly be added character of the coastal sea currents—whether warm or cold—and direction of the prevailing winds.

Faunal conditions depend largely upon humidity as well as upon all zonal factors. The chief cause of a humid climate is, of course, ample precipitation, either rather evenly distributed throughout the year, or else supplemented during the drier season by heavy fogs and dense forests to retard evaporation, while a cool climate is often helpful. Precipitation may be largely dependent upon the position of adjacent mountain masses with respect to the prevailing winds, for, as is well known, moisture-laden air is cooled upon contact with an elevated land mass, and precipitation results; but little moisture will then be left in the clouds for rain in the trans-montanic sections. This fact is beautifully

shown by the humid and heavily forested coast and mountain areas of northwestern Washington, in contrast to the bare, arid plains east of the Cascade range.

Associational temperature is induced by many causes, and although limited in extent it profoundly influences local zonal boundaries. Even associational factors other than temperature may raise barriers to distribution that are insurmountable to many organisms.

Insolation, or the relative amounts of sun and shade received by a species in its habitat, is sometimes of paramount importance. This may be influenced by cloudiness, by the amount and density of surrounding vegetation or by the character of the topographical environment. In illustrating this point, we may mention as extremes the bottom of a deep, narrow, forested gulch, and the top of a warm, bare ridge; the face of a steep north slope, and one facing south. A gully on a north slope may be so situated as never to receive the rays of the sun, while at a certain optimum angle one facing towards the south will receive forty per cent. more solar heat than will a level surface. Hence, zonal boundaries upon the two slope aspects will be found to occur at very different altitudes. Soil conditions are of great importance in influencing the temperature immediately above its surface, and its character helps to control both the amount of evaporation and the degree of moisture which it is capable of retaining. A light-colored soil is considerably cooler, other things being equal, than a dark, rocky one, which will absorb and retain more heat. The importance of the chemical composition as well as the mechanical condition, with amount of humus, acid or alkali, in the soil need be no more than mentioned.

The temperature of the soil and the atmosphere above it is often greatly influenced by near-by cold mountain streams, and in places zonal boundaries may be depressed one or two thousand feet in altitude by this agency. Large snowbanks and glaciers have a similar effect, though usually less pronounced or, rather, more

locally restricted. A forest fire or avalanche, by destroying ground shade with the consequent raising of the soil temperature, will usually cause an area to grow up to plants and trees of the zone immediately below, to be gradually restored, in future years, to its original zonal status. Base level has its effect, for the foot of a mountain mass rising from a plain five thousand feet in altitude will have lower zonal tendencies than will the five thousand foot level of a mountain rising from a plain with an elevation of but one thousand feet, because the higher plain accumulates more heat. Similarly, a large mountain mass is less influenced by the conditions which surround it than is an isolated peak. A steep slope will carry a certain zone to a greater height than will a gentle one, because the former will receive, during the day, more of the warm air arising from the lowlands, and the cold air which descends during the night will flow off more rapidly. However, this rule is often nullified by the steep slope being so situated that it receives less sunlight than the more gentle gradient. These points are finely illustrated on most of the mountains of the southwest. Plants and trees of the Transition Zone often flourish on the bottom of a north-facing canyon, while the Sonoran sagebrush extends a couple of thousand feet higher upon the steep slopes with southern exposures.

Protective cover is important to most of the more retiring forms of active life, and to such it is not only necessary as a screen during their daily foragings, but they must have holes into which they may dart at the approach of danger and safe retreats in which to rear their young. To very few vertebrates is the actual character of the soil of great moment, but there are exceptions, as instanced by the large kangaroo rat, *Dipodomys deserti,* the front feet of which are so weak that it seems able to burrow only in deposits of æolian or other loose sand, and it is useless to expect to find this species in hard soil. Needless to say, character of food, both gen-

eral and specific, is a powerful determining factor of distribution, and with this should be classed not only the manner of feeding but the methods employed in securing sustenance. The search for a favorite food item will even, in time, indirectly change a mammal from a terrestrial to an arboreal type, as it evidently has the tree mouse, *Phenacomys longicaudus,* of the coasts of Oregon and northern California, which, so far as known, feeds exclusively upon the needles of coniferous trees.

The question of enemies, it seems to me, should be given much more weight in distributional problems than it usually receives. This factor may be divided into active and passive enemies. By the latter term is meant competitive forms, as the more robust growth that chokes out a tender seedling, or an organism which, being more adaptable to a variety of conditions, forges ahead of less plastic forms whose habits are competitive. It is the opinion of the writer that such competition constitutes the real remorseless struggle for existence which most species are obliged to carry on in order to survive, rather than their efforts to elude their active enemies. Although these passive enemies are not spectacular and are apparent only after scrutiny by an understanding person, they are, nevertheless, always present and operative.

Active enemies may be divided into irritating and exterminating types, and in certain sections the former may constitute a formidable barrier to dispersal. Few of the larger parasites directly cause death, but the presence of great quantities of aphis, scales, ticks or intestinal worms upon their respective hosts may so handicap a species that it is forced to the wall by the competition of more favored forms. Unusual numbers of horse flies in a mountainous section may so harass stock that they utterly refuse to dwell in such regions.

Exterminating agencies may consist of directly predaceous organisms, such as carnivores which consume the flesh of their victims, or rodents whose presence in

great numbers seriously interferes with the propagation of certain plants. The overstocking of a range with cattle or the presence of a vast colony of prairie dogs may actually extirpate certain grasses in those districts, and hordes of some rodents will prevent reforestation in spots because all tree seeds are eaten as fast as produced. Poisonous plants work great havoc among range stock at times, and although the amount of such devastation among wild forms has seldom or never been investigated, it is doubtless an appreciable factor. In some regions, bacteria and disease, including the smaller parasites, play a most important rôle. The tse-tse fly in portions of Africa has rendered it utterly impossible for certain herbivorous mammals to be kept in the infested districts; the *Stegomyia* mosquito that is instrumental in the spread of yellow fever probably caused the Mayan survivors of this dread disease to abandon the ancient civilization of Yucatan, which was at one time so densely populated, and many ailments, comparatively harmless to white men, who have developed a degree of immunity to them, are largely responsible for the decrease in the numbers within recent years of the more savage peoples. From time to time either totally new bacterial diseases appear or else old ones suddenly acquire new virulence, and throughout the ages, such have undoubtedly killed off certain species from faunal divisions; and it is not at all improbable that during the course of bacterial evolution whole genera, or even families, have been exterminated by this agency.

It seems advisable to append to the present paper a chart, or key, to the factors chiefly responsible for the distribution and restriction of the ranges of living forms, but this is submitted with considerable hesitancy. Most of the factors mentioned are so interdependent upon others that it is merely a matter of personal opinion as to which heading they should be placed under. For instance, it is impossible to decide whether the effect of a cold mountain stream should better be listed under zonal

or associational conditions, for it is operative in both connections. It should be understood, therefore, that the arrangement is only tentative, and that the list has been made to conform to the viewpoint of a vertebrate zoologist.

FACTORS TO BE CONSIDERED IN THE DISPERSAL OF LIFE

Life Types:
- Active Forms.
  - Aquatic.
  - Fossorial.
  - Terrestrial.
  - Arboreal.
  - Volant.
- Sedentary Forms.
  - Character of Habitat.
    - Manner of seeding or reproducing.

Direct Physical Barriers:
- Oceans, rivers, etc. (to land forms).
- Land masses, mountains, etc. (to aquatic forms).
  - Forests, plains, deserts, etc.
    - Protective cover.

Regulation by Temperature:
- Zonal
  - Latitude.
  - Altitude.
  - Proximity to sea.
    - Ocean currents.
  - Prevailing winds.

  → Mean temperature during reproduction.
  → Mean maximum.
  → Mean minimum.
  → Delimiting temperatures (as frost to tender species).

- Faunal.
  - Humidity.
    - Precipitation.
      - Location of near mountain masses, if any.
      - Location of near bodies of water, if any.
- Associational.
  - Degree of insolation.
  - Effects of fires and avalanches.
  - Presence of cold streams or glaciers.
  - Topographical situation.
    - Slope aspect.
    - Slope angle.
    - Base level.
  - Soil.
    - Chemical and mechanical character.

Color.
Moisture.

Food:
General and specific character.
Feeding habits.

Enemies:
Passive (competitive forms).
Active.
Irritating.
Exterminating.
Directly predaceous.
Poisonous foods.
Bacteria, protozoa, etc.

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THE TAPEWORM INFECTION IN WASHINGTON TROUT AND ITS RELATED BIOLOGICAL PROBLEMS

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IN the whole realm of nature man is the only creature whose ailments have seriously occupied the attention of experts. Let a disease break out amongst the human family in some corner of the globe and almost immediately the affliction becomes the target for the trained minds of our ablest pathologists. Not so, however, with the maladies of the lower forms. Man's only interest in them has been one of selfish exploitation, and he has done little to encourage investigations along any other lines except those which bring him immediate monetary returns. It is, therefore, not at all surprising that we possess such meager and fragmentary knowledge concerning disease amongst the lower animals.

It is almost superfluous to say that this attitude must change if we are to intelligently conserve the lower creatures as natural resources. In the last few years we have been hearing a great deal about the conservation of natural resources, and yet very few of us realize the full meaning of conservation. To my mind real conservation implies a thoroughgoing knowledge of the objects to be conserved, coupled with an intelligent application of the factors controlling their preservation. We must possess more knowledge concerning the diseases of the lower animals because it is of prime importance in all conservation programs, in that it may be helpful in preventing great losses of animals which are beneficial to man.

In the state of Washington, as well as in the other states of the Pacific coast, fish afford a natural resource of tremendous importance to the welfare of a large pro-

portion of the citizens, and yet comparatively little is known regarding the diseases which affect these aquatic animals. We become alarmed when the fish begin to die in great numbers, and only then are we in any manner concerned with finding out what ails them.

During the summer of 1919 it was my good fortune to be chosen by the Washington State Fish Commission as a special investigator for the purpose of studying the parasites of the fish in some of the fresh-water lakes and streams of the state of Washington. Prior to undertaking these investigations reports had been coming in to the fish commissioner's office that the fish were dying in the mountain lakes and streams of Kittitas county and, therefore, it seemed advisable to spend most of my time in this region studying the nature and extent of the disease. It is with this epidemic in particular that I wish to deal in the present paper. Incidentally, I desire to point out some of the interesting biological problems with which the question is intimately linked up.

On arriving in Kittitas county the writer found that the people, especially the sportsmen, were very much disturbed about the mortality of their lake trout, for they depended upon these fish to yield them spawn for their county hatcheries. They were particularly distressed about the dying of the trout in Cooper lake, and therefore this lake was the first one which I visited.

Cooper lake is situated in the heart of the Cascade mountains about thirty miles outside of Roslyn. Figures 1–3 show various views of the lake. It is a clear body of water, filled with cut-throat trout. The county game commissioners closed the lake some six years ago in order to obtain a plentiful supply of fish for breeding purposes, and as a result of this the trout have multiplied very rapidly within its waters. For the first few years the results obtained were excellent, but within the last two years the fish commenced to die at an alarming rate, so that all spawning operations had to be abandoned.

An examination of the cut-throat trout of this lake

showed them to be heavily parasitized with larval tapeworms which attack the abdominal cavity. From all appearances these larvæ somewhat resemble those described by Professor Linton in 1889 for the trout of Yellowstone National Park, and, undoubtedly, belong to the genus *Dibothrium* or *Diphyllobothrium,* but are probably of a

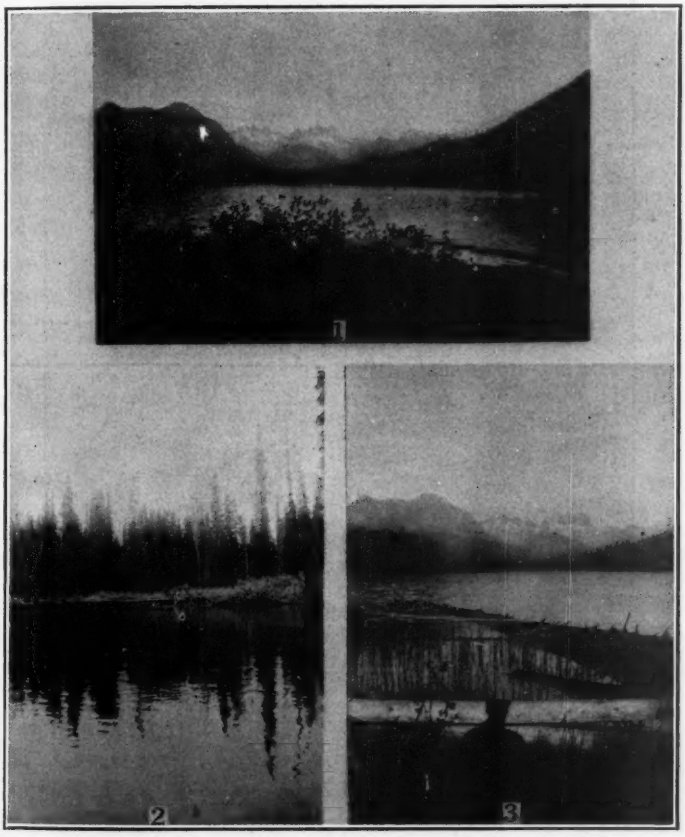

FIG. 1. General view of Cooper lake.
FIG. 2. Region of Cooper lake showing racks, a favorite place for the blue herons.
FIG. 3. Shallow portion of Cooper lake near shore affording an ideal nesting place for fish-destroying birds.

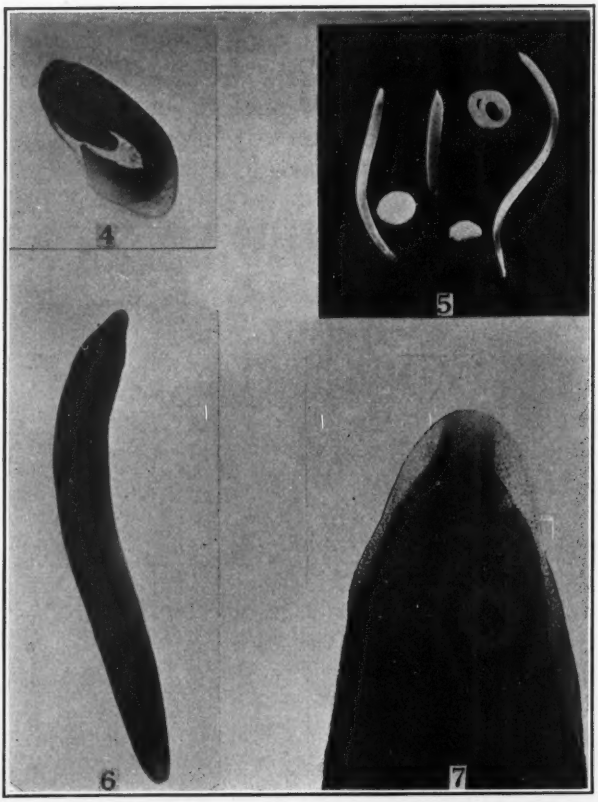

Fig. 4. Tapeworm larva in cyst, × 20.
Fig. 5. Numerous free-boring and encysted tapeworm larvæ, × 3.
Fig. 6. Enlarged photograph of tapeworm larva, × 2?
Fig. 7. Head end of tapeworm larva, × 65.

different species from *Dibothrium cordiceps* Leidy, the ones discussed by Linton. According to Professor A. R. Cooper, of the University of Illinois College of Medicine, to whom specimens of the tapeworm larvæ were sent for identification, "the placing of these larvæ specifically is a matter of the working out of the life histories of the species in question."

The larval tapeworms under consideration may be en-

cysted (Figs. 4 and 5) along the walls of the digestive tract, particularly on the stomach, or they may be found burrowing freely amongst the visceral structures, or within the surrounding muscular walls. In appearance they are translucent, whitish or yellowish-white organisms which may vary from a few millimeters to about twenty millimeters in length. They are long, slender and worm-like in character (Figs. 5 and 6). At the anterior end is the head (Fig. 7), which possesses two lateral slits. This head end is constantly changing its shape in the living specimens, becoming slender and spear-like at one time and stouter and knob-like at another time. The body proper of the larva may undergo periodic contractions and extensions. Covering its entire outer surface are stiff, bristle-like structures which, at first glance, seem to resemble cilia, but which do not possess any independent motion. Posteriorly the body tapers off into a blunt rounded margin (Fig. 6).

The damage done to the fish by these larval tapeworms is considerable. In the first place, the fish lose their healthy appearance, becoming much thinner and paler in hue. The parasitic larvæ undoubtedly produce injurious toxins which interfere with the proper functions of the host. Then, again, the burrowing habits of these parasites injure the tissues of the fish, causing them to become mushy. And finally, secondary infections of a serious sort may develop within the injured portions. As a result of all this damage great numbers of the fish die.

The life history of these larval tapeworms is extremely interesting. Those who are familiar with tapeworm infection know that ordinarily two organisms are necessary for the completion of the life history. The adult tapeworm lives in one animal called the *primary host,* whereas the larval tapeworm dwells in another animal called the *secondary host.* The primary host becomes parasitized by eating the infected portions of the secondary host.

In the case of the tapeworm under consideration it is quite obvious that the trout acts as the secondary host.

The primary host, however, is not definitely known. Professor Linton found that in the case of the infection of the trout of Yellowstone Park the white pelican acted as the primary host, and, in the light of this finding, it is quite probable that some similar fish-eating bird is the primary host of the larval tapeworm under discussion.

While at Cooper lake a canvass was made of the common fish-eating birds which visit the lake, and it was found that the blue heron is the most frequent visitor. Since no pelicans are known to come to the lake, I rather strongly suspect that the blue heron acts as the primary host for the larval tapeworms of the trout. If this should prove to be the case then the life history, in all probability, would be as follows: The adult *Diphyllobothrium* tapeworm develops in the intestinal tract of the blue heron, and when the segments become mature they are periodically passed out with the fæces. These mature segments contain large numbers of developing embryos and if they are deposited in a stream or lake the embryos are swallowed by the fish, in which they develop into the larval tapeworms already described. When a blue heron captures one of these infected fish, the larvæ attach themselves to the bird's intestinal wall and shortly develop into adults capable of carrying on the life cycle.

My visit to Cooper lake convinced me that it was pure folly to entirely close down a lake for more than a year or two. In the first place, closing down a lake makes for a rapid increase of fish so that the available food supply soon becomes inadequate for maintaining all of them, with the result that a fierce struggle for existence ensues, in which many of the weaker, but nevertheless desirable, fish are killed off. Even those which survive in the struggle appear to be starved. Secondly, when a lake is closed its shores afford an ideal, undisturbed nesting place for such fish-destroying birds as the blue heron, kingfisher and the like. These birds not only destroy large numbers of fish, but they may be the means of disseminating parasitic infections. And lastly, in the light

of the experience in other states, it is a useless waste of money to depend on the fish in a large natural body of water for spawn, because it is very difficult to control the factors which insure success.

Two other mountain lakes were next visited: Lost lake on Roaring creek, near Keechelus, and Fish lake.

Lost lake is stocked with eastern brook and cut-throat trout, with the former predominating in much larger numbers. The lake has been closed for several years and was utilized by the county game commissioners as a place for obtaining eastern brook-trout spawn. From this lake seventy-six brook-trout and two cut-throat trout were examined, and with the exception of two brook-trout all the fish were found to be clean and healthy. The two exceptions mentioned were each parasitized with a single larval tapeworm cyst.

The situation at Lost lake seemed very striking as well as significant, and it suggested the possibility that perhaps the brook-trout are more resistant and immune to the parasitism of the tapeworm larvæ. At any rate, this is worth while testing out much more thoroughly.

One other point which the trip to Lost lake strengthened was in regard to what has already been said concerning the food supply of a closed lake. The fish in this lake, although they were nearly all healthy, were nevertheless very thin. The most prominent parts of them were their heads. In two cases the fish were so hungry that they captured field mice which probably attempted to swim across the lake. These were found partially digested within the stomachs of the fish.

At Fish lake one hundred and nine trout were caught, mainly of the cut-throat species, and a careful examination revealed the fact that they were all healthy and clean. There wasn't a single indication of tapeworm infection. Fish lake was an open body of water and this probably accounts for the healthy state of the fish. When sportsmen can get into a stream they are a source of disturbance to the blue heron and other fish-eating birds, and.

therefore, these birds are prevented from nesting along the shores, thereby protecting the stream from becoming infected with the tapeworm disease.

At the termination of the investigations in Kittitas county, the writer made the following specific recommendations to the county game commissioners:

1. Not to close lakes for more than a short time, say a year or two, and only for the purpose of conserving the fish. When a lake is closed for many years the normal multiplication of fish is such that the food supply within the lake is greatly diminished, resulting in a starvation process. Furthermore, unless adequate watch is maintained, the heron and other fish-destroying birds will live along the shores of these closed lakes and serve as a constant source of infection for the fish.
2. Not to depend on the closed lakes for spawn, but instead to develop a hatchery or a series of hatcheries with numerous outdoor ponds where they can place many of the healthy trout from Lost and Fish lakes, which will give them a constant supply of healthy spawn. They will not only save money by such a project, but their efforts will not be wasted.

After the completion of the above studies the writer examined fish from various places in King county, in which he has found the same larval tapeworm infection. Numerous cut-throat trout of Klause lake near Snoqualmie falls were examined and found to be heavily parasitized. Also, the silver salmon and the so-called red fish or silver trout (which are nothing more than land-locked sockeye salmon) were found to be heavily infected with the same parasites. The striking thing about the parasitism of these last-named fish was that they were more heavily parasitized than any of the fish previously examined in which the tapeworm larvae were found to dwell.

The observations recorded in the present paper make it obvious that a good many of our fish and game cultural practices are utterly wasted because we are ignorant of those factors which ought to insure success. What is urgently needed in the state of Washington as well as in the neighboring states of the Northwest is a series of "Biological Surveys" for the purpose of studying and mapping out the various ecological factors of the regions in which fish or game are to be planted. We ought to know a good deal about such factors as available food supply, oxygen content, temperature variations, predatory and parasitic organisms, etc., of a place before any kind of animals or plants are introduced into it. Knowing these conditions we can then intelligently fit each organism into that particular environment where it will thrive best. But without this knowledge we are simply groping in the dark and are powerless to do any real good.

MIGRATIONS AND AFFINITIES OF THE FOSSIL PROBOSCIDEANS OF EURASIA, NORTH AND SOUTH AMERICA, AND AFRICA

(Sixth contribution on the evolution of the Proboscidea)

DR. HENRY F. OSBORN

American Museum of Natural History

Dr. Hikoshichiro Matsumoto, of the Tôhoku Imperial University, Sendai, Japan, has recently been studying the Fayûm collections of primitive proboscideans and hyracoids in The American Museum of Natural History, followed by a visit to the British Museum where he has been making comparisons with the types of these mammals, described by Dr. C. W. Andrews in his series of papers beginning in 1901. In 1918 Doctor Matsumoto[1] published a series of five papers on the elephants, turtles, sirenians, cervids, and bisons of Japan compared with those of India. He pointed out that the Japanese archipelago was an integral part of the continent from the beginning of the Miocene to the middle of the Pleistocene, and that the period of separation seems to have dated from the recent Pleistocene. Consequently its relations with the animal life of southern China and with the more distant peninsula of India are very close.

The ancient Japanese proboscideans are chiefly of three kinds, of which the most numerous are the forest-living stegodonts, closely related in their specific phases to the stegodonts of China, such as the species *Stegodon sinensis*. There also occurs in the early Pleistocene the

[1] 1. "On a New Archetypal Fossil Elephant from Mt. Tomuro, Kaga." 2. "On a New Fossil Trionyx from Hokkaido." 3. "A Contribution to the Morphology, Palæobiology and Systematic of *Desmostylus*." 4. "On a New Archetypal Fossil Cervid from the Prov. of Mino." 5. "On Some Fossil Bisontines of Eastern Asia." *Sci. Rep. Tôhoku Imp. Univ.*, Sec. Ser. (Geology), Vol. III, No. 2.

great *Loxodon antiquus namadicus,* the straight-tusked elephant, which ranged all over southern Eurasia and probably arose originally in the African continent.

In the early formations, such as the Middle Pliocene of Tomuro, Kaga, we meet the *Elephas auroræ,* regarded by the author as an intermediate type between the stegodonts of the Upper Pliocene of India and *Elephas planifrons,* which in turn is related to the true mammoths (*Elephas primigenius*) and wandered all over southern Europe in Upper Pliocene time, namely, Bessarabia, Austria, and southern France. In still earlier deposits, such as the Upper Miocene of Kuji, occurs a mammal which the author refers to *Stegodon latidens,* an ancestral stegodont of Burma, India. In the Lower Miocene of the Province of Mino occurs a form very similar to the *Trilophodon angustidens* of the Middle Miocene of France, ancestral to all the long-jawed proboscideans.

The *Stegodon* itself is peculiar to India, China, Japan, and the larger islands of the Malayan archipelago, such as Sumatra, Java, and Borneo. The author notes that there is a marked difference between the sexes, so that the stegodonts of each geologic period seem to have received two specific names, one applied to the female, the other to the male form. Among these couples are *S. Cliftii-bombifrons,* dating from the Upper Pliocene and from the Lower Pliocene of India; *S. ganesa-insignis,* dating from the Upper Pliocene and from the Postpliocene of the same area; *S. sinensis-orientalis,* dating from the same strata of China and Japan; *S. airawana-trigonocephalus* from the Postpliocene of Java. This sex dimorphism is very marked, especially in the great disparity of size of the upper tusks, which are much smaller and more slender in females than in males. This tusk structure in turn affects the entire form of the head.

The *Bison occidentalis* of Japan seems to spring from the *B. sivalensis* of the Upper Pliocene of India. It is similar in fact to the bison found in the ancient Pleistocene of Kansas, in the basin of the Ohio River, in Alaska,

and in the region of the Yenisei River in Siberia. According to the author, in the Transbaikal region the same species occurs in association with the giant woolly rhinoceros (*Diceros antiquitatis*), with the hairy mammoth (*Elephas primigenius*), and with the heavy-horned bison (*Bison crassicornis*).

Quite a different order of distribution has the remarkable *Desmostylus*, a sirenian or sea cow peculiar to the coasts of the Pacific Ocean, first described from the California coast many years ago by Professor Marsh and more recently recorded from Japan. The Japanese species is much more specialized and of larger size than the forms occurring on the Oregon and California coasts, which points to a general migration from east to west, that is, from the Orient to the Pacific coast of North America.

From this series of papers we are able to draw up the following table showing the principal distribution of the species of mammals in the descending order of the deposits in Japan:

Postpliocene of Shôzu-shima (Sanuki): *Stegodon sinensis, S. orientalis, Loxodon antiquus namadicus, Bison occidentalis, Cervus* (*Sika*) cf. *nippon.*

Upper Pliocene of Ikadachi-mura (Omi): *Stegodon sinensis, S. orientalis, Buffelus* sp.

Middle Pliocene of Tomuro (Kaga): *Elephas auroræ.*

Upper Miocene of Kuji (Hitachi): *Stegodon* cf. *latidens.*

Middle Miocene of the Provinces of Teshio, etc.: *Desmostylus japonicus.*

Lower Miocene of the Province of Mino: *Trilophodon* cf. *angustidens, Teleoceras* sp., *Amphitragulus minoënsis.*

The present researches of Doctor Matsumoto on the rich Fayûm collections of the American and British Museums have enabled him to draw an important distinction in northern Africa between the true forest-living mastodons, which appear to be directly descended

from the genus *Palæomastodon* of the Fayûm, and the long-jawed mastodons, which appear to be directly descended from *Phiomia* of the Fayûm. This interesting discovery, which was partly anticipated in Doctor Andrews's own papers, enables us to trace the American mastodon far back into Upper Eocene times of northern Egypt.

In this connection may be mentioned also a series of five papers[2] by the present reviewer on the "Evolution, Phylogeny, and Classification of the Proboscidea" which have appeared successively since 1918. The writer is attempting to give an iconographic revision of the entire group of proboscideans, including the progenitors of Africa and Eurasia and the highly developed descendants of North and South America, which together make up the most remarkable family history of which we have record.

In 1900 Osborn predicted that the source of the mammalian order of the Proboscidea would probably be discovered in Africa. In 1901 Beadnell and Andrews revealed, through the Geological Survey of Egypt, the rich fauna of the Fayûm, southwest of Cairo, in which were found the remains of three proboscidean genera, named by Andrews *Mœritherium, Palæomastodon, Phiomia,* and confirmed by subsequent exploration and research to be the oldest proboscideans thus far known. Animals similar to *Mœritherium* and *Phiomia* have since been reported by Pilgrim in southern Asia. These animals are

[2] The first paper in this series is entitled "A Long-jawed Mastodon Skeleton from South Dakota and Phylogeny of the Proboscidea," *Bull. Geol. Soc. Amer.*, XXIX, March, 1918; the second paper, "Evolution, Phylogeny, and Classification of the Proboscidea," *Amer. Mus. Novitates* No. 1, January 31, 1921 (Osborn, 1921. 515); the third paper, "First Appearance of the True Mastodon in America," *Amer. Mus. Novitates* No. 10, June 15, 1921; the fourth paper appears in the *Bulletin* of the Geological Society of America, under the title "Evolution, Phylogeny, and Classification of the Mastodontoidea"; the fifth paper, "Adaptive Radiation and Classification of the Proboscidea," was read before the National Academy of Sciences, April 26, 1921. The present is the sixth paper. The Iconographic Type Revision will form one of the Memoirs of the American Museum of Natural History.

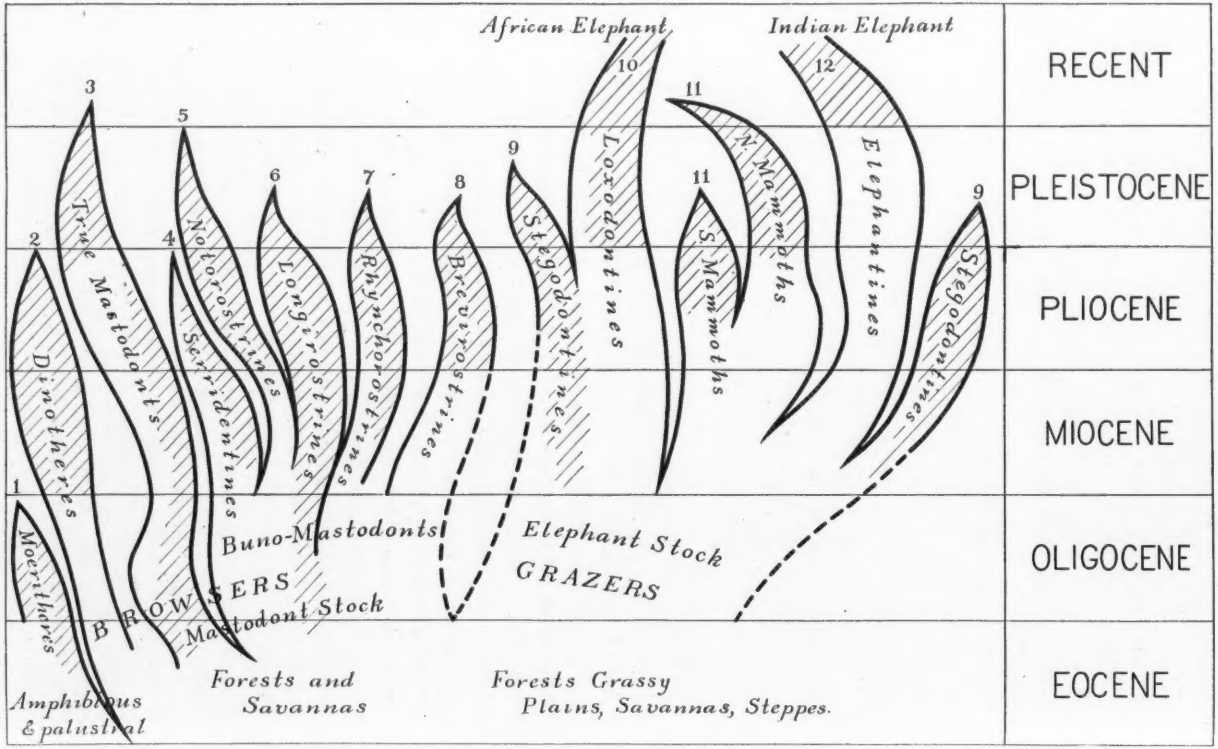

FIG. 1. Diagram showing the present theory as to the adaptive radiation of the Proboscidea. June, 1921.

now found to belong respectively to three distinct lines of the Proboscidea, namely, the mœritheres, the true mastodonts, the long-jawed bunomastodonts, as indicated in black on the accompanying diagram. They point, however, to a long antecedent origin and radiation. This is part of the evidence for an ancient adaptive radiation process by which it now appears that the proboscideans, like other hoofed mammals, were broken up into several great primary stocks way back in Eocene times, namely:

An *amphibious* stock, adapted to rivers and swamps, of limited migration (= *Mœritherium, Dinotherium*).
A *mastodont* stock, adapted to forests and savannas, of wide migration (= mastodonts, trilophodonts).
A *stegodon-elephant* stock, adapted to southern forests, to grassy plains, to savannas and steppes, of wide migration (= *Stegodon, Loxodon, Elephas*).

These primary stocks gave off from two to six branches each, so that the Proboscidea as a whole are not two branched (*i.e.,* mastodonts and elephants), as formerly supposed, but many branched or *polyphyletic.* The forest and savanna browsers and the grazers of the plains and steppes were the long distance travelers and from an African or Asiatic center in Eocene times they reached in the Middle and Upper Miocene all the continents of the world except Australia, while the amphibious forms remained in Africa and southern Eurasia. Certain of these branches, like the true mastodons, are of very great geologic antiquity. Intelligent, independent, well defended, resourceful, adaptive, we find eleven very distinct branches of proboscideans persisting into Upper Pliocene times, five of the least hardy of which became extinct during the colder conditions of the Lower Pleistocene. The known lines of evolution are shaded on the accompanying diagram; the unknown are left in white. The theoretic adaptive radiation may be expressed in a formal classification as follows:

Amphibious and swamp-living stock

I. MŒRITHERIOIDEA (Mœritheres)

1. Mœritheriini,[3] amphibious or swamp-living forms known in the Upper Oligocene of Africa.

II. DINOTHERIOIDEA (Dinotheres)

2. Dinotheriini,[4] large amphibious forms frequenting the rivers of southern Eurasia throughout the Miocene to the close of the Pliocene.

Forest and savanna grazers

III. MASTODONTOIDEA (Mastodonts and Bunomastodonts)

MASTODONTIDÆ or "true mastodonts," including the subfamilies

3. Mastodontinæ, springing from *Palæomastodon* of the Oligocene of North Africa, and terminating with *Mastodon americanus* of the Pleistocene forests of North America; grinders lophodont, lacking trefoils.

4. Serridentinæ,[5] first known in the Middle Miocene of France and Switzerland, spreading over into India and North America; lacking the trefoils.

BUNOMASTODONTIDÆ, the bunomastodonts, springing from forms similar to the *Phiomia* of North Africa and separating into four main divisions:

6. Longirostrinæ, typical long-jawed bunomastodonts arising in North Africa (*Phiomia*), spreading all over southern Europe, Asia, and North America.

5. Notorostrinæ, a special branch entering the Andean region of South America and spreading over the South American continent, distinguished by the loss of the lower tusks and the abbreviation of the jaw.

7. Rhynchorostrinæ, beaked bunomastodonts, known only in the southern United States and northern Mexico, with powerful downturned upper and lower tusks.

8. Brevirostrinæ, short-jawed bunomastodonts, which imitate both the true mastodonts and the elephants in the abbreviation of the lower jaw and the early loss of the inferior tusks.

[3] Herluf Winge, 1906, p. 172.

[4] *Ibid.*

[5] It is a question whether this subfamily is nearest the Mastodontidæ, with which its members are generally placed by European paleontologists.

These animals wandered all over Europe, Asia, and western North America.

IV. ELEPHANTOIDEA (the Elephant stock)

9. Stegodontinæ, the original members of which were doubtless ancestral to all the higher elephants, persist as an independent branch into the Lower Pleistocene of eastern Asia.
10. Loxodontinæ, embracing the great African division of the elephants beginning with varieties of the *Loxodon antiquus* of the Upper Pliocene, which wandered all over southern Eurasia and radiated widely over Africa.
11. Mammontinæ, including (*a*) the Southern Mammoths (*Elephas planifrons* of India and *E. meridionalis* of Europe), from which is derived *E. imperator* of North America, and (*b*) the Northern Mammoths, which probably include *E. columbi* and the widespread *E. primigenius* of the northern steppes; (?) tetradactyl pes.
12. Elephantinæ, the true elephants (*E. indicus* of India), which do not appear until the Upper Pleistocene; pentadactyl pes.

This twelve-fold branching of the proboscideans is similar to the adaptive radiation which the author has traced in the evolution of the horses, of the rhinoceroses, and of the titanotheres, carrying the fundamental lines of separation back to the Middle Miocene as the most recent date, and to the Middle or Lower Eocene as the most remote date. It will be observed from the diagram (Fig. 1) that the shaded areas represent those proboscidean phyla of which remains have been discovered. The large unshaded area includes the entire Oligocene, Miocene, and Lower and Middle Pliocene history of the Elephantidæ which is still unknown but which is likely to be revealed at any time by discoveries both in Africa and in central Asia. A very striking fact is that the early member of the Elephantoidæ, the *Elephas planifrons* of the Upper Pliocene of India and the apparent ancestor of the mammoths, is now antedated in geologic time and in its transitional structure by the *Elephas auroræ* (*i.e.*, of the rising sun region) of Japan.

BOOKS AND LITERATURE

The Conservation of the Wild Life of Canada. By Dr. C. Gordon Hewitt, late Dominion Entomologist and Consulting Zoologist. New York: Charles Scribner's Sons, 344 pp., illustrated.

This book was in manuscript before the untimely death of Doctor Hewitt, February, 1920, and has been prepared for publication by his wife. Mrs. Hewitt has also written a beautiful preface which can perhaps be fully appreciated only by those who had the rare good fortune to count Hewitt as a personal friend.

To get the proper perspective on this book, one should know that Doctor Hewitt was a zoologist of broad training. Previous to coming to Canada he had worked not only on insects but also on several problems on birds and their control of insect pests. The record of his work as Dominion Entomologist from 1909 until his death is a brilliant one. Throughout this period he was frequently consulted regarding various zoological problems which came before the Advisory Board on Wild Life Protection and in 1916 he was appointed Dominion Consulting Zoologist which broadened his official interest. The work recorded in the book under discussion was done chiefly during the last four years of his life. For so busy a man to undertake a task of this size and to cover the field so well in so short a time is an enviable accomplishment.

The reading of this book is like a trip to the North Woods, but with a scientist as companion rather than a record-breaking hunter of big game. Although the title might properly include fur-bearing animals and other natural groups, the discussion is chiefly limited to the larger wild mammals and birds of Canada. The information regarding the present distribution and abundance of the several species is accumulated from many sources and constitutes a valuable inventory of the remaining but diminishing resources of the Dominion. As might be expected of one who understands the dangers of promiscuous and ignorant hunting and who appreciates wild life, the dangers and economic loss of unrestricted shooting are constantly set forth, and the results of inadequately controlled slaughter in the United States

are used as an ignoble example. It is not too late in Canada to profit by mistakes south of the boundary, and Doctor Hewitt's book should serve as a timely warning.

Considerable progress has already been made in establishing government and private reserves in Canada and the record of this movement as given in this book is one of the most valuable features of the work. The author took a lively interest in this movement, and in efforts to conserve the wild life of the Dominion he did everything possible, from revising the game laws of the Northwest Territories to the instruction of Boy Scouts in bird protection. Previous to his work the game laws of the Northwest Territories had not been revised for many years, and he succeeded in the difficult task of getting through a revision that is a great improvement over the former regulations. His successful effort to bring about the Migratory Birds Treaty between the United States and Canada was an accomplishment of high order. There were, of course, other earnest men on both sides of the boundary who assisted in this work, but to the author of this book fell some of the most aggravating and ability-testing tasks. If the full history of this effort is sometime written, Doctor Hewitt's part will appear as a large one.

The discussion of the periodic fluctuations of Canadian fur-bearing animals in Chapter IX is perhaps the best example of scientific method in the book. These fluctuations have long attracted the attention of scientific and commercial men and they are here discussed from abundant data and from a biological point of view.

This posthumous book is an additional monument to the scientific skill and personal abilities of the author. It should serve as a valuable warning to Canadians and will be of value to readers everywhere in giving a summary of the resources of the Dominion in one of its most interesting and economically valuable assets. Because of the wide interest in big game it should attract temporarily or permanently to Canada those who retain a wholesome love for the outdoors.

E. F. PHILLIPS

SHORTER ARTICLES AND DISCUSSION

THE PROBABILITY ESTABLISHED BY A CULTURE OF GIVEN SIZE THAT A MATING IS CAPABLE OF PRODUCING ONLY DOMINANT INDIVIDUALS

To distinguish individuals heterozygous from those homozygous for a given dominant factor is a matter of mere inspection when the simplex condition is somatically distinct from the duplex condition, as is the case with the mottling factor in the Adzuki Bean.[1] Generally, however, the degree of dominance is such that a breeding test must be resorted to in order to distinguish these two types. A homozygous dominant will breed true for the character whether selfed or back-crossed to the recessive, whereas a heterozygous individual will give 3 : 1 and 1 : 1 ratios respectively when similarly treated. The common breeding practice is to consider the parent homozygous when, if selfed or back-crossed, it fails to produce any recessive individuals in a reasonably large number of offspring.

Just what is to be considered an adequately large number of offspring has in the past been determined by the personal judgment of the individual investigator, and the difficulty of obtaining offspring in large numbers. There has been no general agreement based on mathematical considerations, probably because large numbers of offspring have not been found necessary in order to distinguish a homozygous dominant from a heterozygous parent producing such ratios as 3 : 1 and 1 : 1. The need of a statistical criterion of what is an adequately large number of offspring was realized when it became necessary in tetraploid races of the Jimson Weed (*Datura Stramonium*) to distinguish between matings which should produce only dominant purple offspring and those which should produce a 35 : 1 ratio of purples to whites. In distributions which are so asymmetrical as those given by sampling from the 35 : 1 ratio, we are hardly justified in using the ordinary theory of probable errors. Special tables have, therefore, been computed for use in work under way at the Station for Experimental Evolution. Since other investigators will probably meet with the need for similar criteria, it seems worth while to give tables showing the number of offspring

[1] *Jour. Hered.*, 8, 125–131, Fig. 10, 1917.

which should be considered in order to distinguish matings which should give all dominant individuals from those which may produce recessives.

The theory is of course quite simple. It is assumed that the expected ratio of dominant to recessive is known, and is $p : q$, where $p + q = 1$. The distribution of the chances of obtaining dominant and recessive individuals in the frequencies $n : 0$, $(n—1) : 1$, $(n—2) : 2$, etc., when n individuals are grown is $(p + q)^n$. To ascertain the probability of securing all dominant individuals in a culture which should show a definite ratio of dominant to recessive offspring we have merely to table p^n against n. If this value is very small, it is reasonable to assume that in practice a culture of n individuals all of the dominant type represents a parent or parents capable of producing only offspring of the dominant type. Thus, for example, if seeds which should produce dominant and recessive individuals in a 5 : 1 ratio were sown, a culture of 35 all dominant individuals should be obtained only 17 times in 10,000. Hence, if a sowing is made to distinguish between a mating capable of producing only dominants and one which should give recessives in a 5 : 1 ratio, and there results a culture of 35 individuals all of the dominant type, it is altogether reasonable to assume that the mating in question is incapable of producing recessives.

Tables have been formed to include the 3 : 1 and 1 : 1 ratios familiar in ordinary disomic inheritance, the 2 : 1 and 8 : 1 ratios found in trisomic inheritance in the mutant Poinsettia, and the 5 : 1, 11 : 1, and 35 : 1 ratios found in tetraploids in *Datura*. Some of these ratios are suggested by published data on *Œnothera Lamarckiana* and *Primula sinensis*, and will probably be found ultimately by those studying other forms.

The tables enable one to decide how large a culture is necessary on a probability basis. If it is felt that only 1 chance in 1,000 of the mating being capable of producing a recessive is sufficient evidence that the culture represents only dominants, then, to distinguish a mating which can produce only dominants from one which should give a 1 : 1 ratio, a culture of at least 10 individuals is necessary. If the 3 : 1 ratio is the one in question, then 24 individuals are necessary; while if a 35 : 1 ratio is considered, 244 individuals are required. In other words, cultures of 10, 24 and 244 individuals are of equal value in distinguishing matings which should produce only dominants from those which

should give, respectively, 1 : 1, 3 : 1, and 35 : 1 ratios of dominants to recessives.

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JOHN BELLING,
J. ARTHUR HARRIS.

TABLE I

VALUES OF p^n FOR 1 : 1, 2 : 1, 3 : 1, AND 5 : 1 RATIOS

N	1 : 1	2 : 1	3 : 1	5 : 1	*N*	3 : 1	5 : 1
1....	.5000	.6667	.7500	.8333	19	.0042	.0313
2....	.2500	.4444	.5625	.6944	20	.0032	.0261
3....	.1250	.2963	.4219	.5787	21	.0024	.0217
4....	.0625	.1975	.3164	.4823	22	.0018	.0181
5....	.0313	.1317	.2373	.4019	23	.0013	.0151
6....	.0156	.0878	.1780	.3349	24	.0010	.0126
7....	.0078	.0585	.1335	.2791	25	—	.0105
8....	.0039	.0390	.1001	.2326	26	—	.0087
9....	.0020	.0260	.0751	.1938	27	—	.0073
10....	.0010	.0173	.0563	.1615	28	—	.0061
11....	—	.0116	.0422	.1346	29	—	.0051
12....	—	.0077	.0317	.1122	30	—	.0042
13....	—	.0051	.0238	.0935	31	—	.0035
14....	—	.0034	.0178	.0779	32	—	.0029
15....	—	.0023	.0134	.0649	33	—	.0024
16....	—	.0015	.0100	.0541	34	—	.0020
17....	—	.0010	.0075	.0451	35	—	.0017
18....	—	—	.0056	.0376	36	—	.0014

TABLE II

VALUES OF p^n FOR 8 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
1.....	.3079	.2737	.2433	.2163	.1922	.1709	.1519	.1350	.1200	.1067
2.....	.0948	.0843	.0749	.0666	.0592	.0526	.0468	.0416	.0370	.0329
3.....	.0292	.0260	.0231	.0205	.0182	.0162	.0144	.0128	.0114	.0101
4.....	.0090	.0080	.0071	.0063	.0056	.0050	.0044	.0039	.0035	.0031
5.....	.0028	.0025	.0022	.0019	.0017	.0015	.0014	.0012	.0011	.0010

TABLE III

VALUES OF p^n FOR 11 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
1.....	.4189	.3840	.3520	.3227	.2958	.2711	.2485	.2278	.2088	.1914
2.....	.1755	.1609	.1475	.1352	.1239	.1136	.1041	.0954	.0875	.0802
3.....	.0735	.0674	.0618	.0566	.0519	.0476	.0436	.0400	.0366	.0336
4.....	.0308	.0282	.0259	.0237	.0217	.0199	.0183	.0167	.0154	.0141
5.....	.0129	.0118	.0108	.0099	.0091	.0083	.0077	.0070	.0064	.0059
6.....	.0054	.0050	.0045	.0042	.0038	.0035	.0032	.0029	.0027	.0025
7.....	.0023	.0021	.0019	.0017	.0016	.0015	.0013	.0012	.0011	.0010

TABLE IV

VALUES OF p^n FOR 35 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
3.....	.4295	.4176	.4060	.3947	.3837	.3731	.3627	.3526	.3428	.3333
4.....	.3241	.3151	.3063	.2978	.2895	.2815	.2737	.2661	.2587	.2515
5.....	.2445	.2377	.2311	.2247	.2184	.2124	.2065	.2007	.1952	.1897
6.....	.1844	.1793	.1744	.1695	.1648	.1602	.1558	.1515	.1473	.1432
7.....	.1392	.1353	.1316	.1279	.1244	.1209	.1175	.1143	.1111	.1080
8.....	.1050	.1021	.0993	.0965	.0938	.0912	.0887	.0862	.0838	.0815
9.....	.0792	.0770	.0749	.0728	.0708	.0688	.0669	.0651	.0632	.0615
10.....	.0598	.0581	.0565	.0549	.0534	.0519	.0505	.0491	.0477	.0464
11.....	.0451	.0439	.0426	.0414	.0403	.0392	.0381	.0370	.0360	.0350
12.....	.0340	.0331	.0322	.0313	.0304	.0296	.0287	.0279	.0272	.0264
13.....	.0257	.0250	.0243	.0236	.0229	.0223	.0217	.0211	.0205	.0199
14.....	.0194	.0188	.0183	.0178	.0173	.0168	.0164	.0159	.0155	.0150
15.....	.0146	.0142	.0138	.0134	.0131	.0127	.0123	.0120	.0117	.0113
16.....	.0110	.0107	.0104	.0101	.0098	.0096	.0093	.0091	.0088	.0086
17.....	.0083	.0081	.0079	.0076	.0074	.0072	.0070	.0068	.0066	.0065
18.....	.0063	.0061	.0059	.0058	.0056	.0055	.0053	.0052	.0050	.0049
19.....	.0047	.0046	.0045	.0044	.0042	.0041	.0040	.0039	.0038	.0037
20.....	.0036	.0035	.0034	.0033	.0032	.0031	.0030	.0029	.0029	.0028
21.....	.0027	.0026	.0025	.0025	.0024	.0023	.0023	.0022	.0021	.0021
22.....	.0020	.0020	.0019	.0019	.0018	.0018	.0017	.0017	.0016	.0016
23.....	.0015	.0015	.0015	.0014	.0014	.0013	.0013	.0013	.0012	.0012
24.....	.0012	.0011	.0011	.0011	.0010	.0010	.0010	.0010	.0009	.0009

LINKAGE BETWEEN BRACHYSM AND ADHERENCE IN MAIZE

ADHERENCE first appeared in the second generation of a brachytic x Boone Co. White hybrid and seemed to be linked closely with normal stature.[1] Subsequent progenies indicated that there was no very close linkage between these characters and possibly none at all.[2] The relationship of these two interesting characters has been tested now in more detail and it seems certain that their genes are located on the same chromosome.

A cross was made between a non-adherent brachytic plant and an adherent plant of normal stature, both plants being segregates in the F_2 of the brachytic-Boone hybrid. The first generation segregated with respect to the brachytic culms, approximately half the plants being of normal stature, but none exhibited a tendency toward adherence. From the behavior of the F_1 plants it is apparent that the adherent parent of the cross was heterozygous with respect to the brachytic character.

[1] Kempton, J. H., "A Brachytic Variation in Maize," U. S. Dept. of Agri. *Bull.* 925, Feb., 1921.

[2] Kempton, J. H., "Heritable Characters in Maize V. Adherence," *Journal of Heredity*, Vol. XI, No. 7, Sept.–Oct., 1920.

Three F_1 plants of normal stature were self-pollinated and three were back-crossed on the double recessive (adherent-brachytic). The six ears were planted separately at Arlington, Virginia, but the resulting F_2 populations were not as large as could be desired.

The combined self-pollinated progenies gave the following distribution:

No. Plants				Per Cent.			
Nor.	Br.	Ad.	Br.-Ad.	Br.	Ad.	Crossover	Q.
217	91	85	4	23.9	22.4	22.2 ± 3.4	.798 ± .06

and the plants of the combined back-crossed progenies are distributed as follows:

Nor.	Br.	Ad.	Br.-Ad.	Br.	Ad.	Crossover
86	188	178	71	49.5	47.6	30.0 ± 1.35

These distributions clearly indicate that crossing over between these two factors occurred in from 20 to 30 per cent. of the gametes.

Additional evidence of linkage between these characters is afforded by the second generation of a cross between an adherent plant of normal stature and a ramose-brachytic plant. The F_1 of this cross was normal with respect to all three characters, and they all reappeared in the progenies of the second generation. Five F_1 plants were self-pollinated and the resulting ears planted separately. Unfortunately in most of the F_2 progenies there is a deficiency of adherent plants and for the combined progenies the departure below the expected 25 per cent. is 7.8 ± .87, a deviation too large to be ascribed to chance. Whether this deficiency represents seedling mortality is not known, but at the time the plants were classified many of the progenies contained late plants strikingly smaller and weaker than their mature sisters. Some of these plants consisted of a small cluster of grasslike leaves with inflorescences hardly developed beyond the embryonic stage. Such plants could not be classified with respect to adherence, though in many cases it was possible to determine satisfactorily whether they were ramose or brachytic. With respect to these last two characters

the small late plants approximated the familiar 9–3–3–1 grouping. If the assumption is made that all these late plants were adherent, the percentage of adherent plants in most of the progenies would then approximate the expected. For the present analysis of the relationship of brachytic and adherent, the low percentage of adherent plants is not important, since the percentage of crossovers can be determined from the ratio of normal to brachytic plants or by the use of Yule's Coefficient of Association.[3]

Combining the five progenies the distribution of plants is as follows:

NUMBER OF PLANTS

Nor.	Ad.	Ra.	Br.	Ad.-Ra.	Ad.-Br.	Ra.-Br.	Ad.-Ra.-Br.	Small Plants
361	135	117	193	18	3	57	2	61

PER CENT.

Ad.	Ad. and Small Plants	Ra.	Br.
17.8 ± .87	23.2 ± .92	21.9 ± .94	27.9 ± 1.0

PER CENT. OF CROSSOVERS

Ad.-Ra.		Ad.-Br.		Ra.-Br.	
Q.	%	Q.	%	Q.	%
37 ± .06	38.5 ± 1.8	.886 ± .03	16.8 ± 1.9	.05 ± .06	49.9 ± 0.4

It is seen that the progenies of this hybrid indicate about 17 per cent. of crossing over while the three self-pollinated progenies of the other hybrid, involving brachytic and adherent, indicate 22 per cent. and the back crosses 30 per cent. It seems inadvisable to combine the self-pollinated progenies from the two hybrids to arrive at a single figure for the percentage of crossovers since the degree of crossing over between two factors often varies greatly in different progenies. It seems certain from these two hybrids that these two characters are located in the same chromosome separated by a distance varying from 18 to 30 units, thus making a linkage series of brachytic, adherent and pericarp color.

[3] Yule, G. Udney, "On the Association of Attributes in Statistics," *Phil. Trans. Roy. Soc.*, London, S. A., Vol. 94, pp. 257–319, 1900.

The progenies of the brachytic-adherent-ramose hybrid furnish evidence that the ramose character may belong to the same linkage series, though the linkage is rather loose.

Although the tassels of ramose plants are much larger than those of normal plants and it seemed not unreasonable to expect adherent-ramose tassels to present a large thickened mass, nothing of the sort was found and the ramose-adherent plants could be separated from the normal-adherent plants only by examining the ears.

White and colored seeds were planted separately, but the percentage of the three characters are essentially alike, as is shown by the following figures indicating that all three are independent of one of the aleurone factors:

	% Adherent	% Ramose	% Brachytic
White seeds planted	16.4 ± 1.65	24.3 ± 1.92	31.5 ± 2.04
Colored seeds planted.....	18.3 ± 1.00	21.1 ± 1.07	27.9 ± 1.17
Difference.....	1.9 ± 1.93	3.2 ± 2.2	3.6 ± 2.34

J. H. KEMPTON

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A GENE FOR THE EXTENSION OF BLACK PIGMENT IN DOMESTIC FOWLS[1]

THE results of recent experiments on the inheritance of plumage colors in fowls indicate that varieties in which black pigment extends to all or nearly all of the plumage (*e.g.*, self black) differ by one dominant autosomal gene from varieties in which black pigment is restricted to the hackle, flight and tail feathers (*e.g.*, Columbian and buff varieties). This gene has been called "extension of melanic pigment" and has been assigned the symbol E^m.

The evidence is derived from reciprocal crosses between Black Orpington and Columbian pattern (Light Brahma) fowls. Whichever way the cross is made the F_1 chicks are all black in the down. As adults, the males from the reciprocal crosses are alike. They are black with white-bordered hackles and saddle feathers; white-bordered and splashed or stippled wing coverts and narrow white borders on the upper breast feathers. They resemble fairly typical Dark Brahma or Duckwing males.

[1] Contributions in Poultry Genetics, Storrs Agr. Experiment Station.

The females from the reciprocal crosses are unlike in adult plumage. From the cross of Black male by Columbian female, the daughters are self black. From the cross of Columbian male by Black female, the daughters are black with white borders on the feathers of head, hackle, and upper breast. They resemble the pattern known as Birchen.

When backcrossed with Columbian females, the F_1 males have produced black, Columbian and *buff* chicks in the ratio of 4: 3: 1; or approximately equal numbers of chicks with extended and restricted black pigment. When crossed with buff females the same F_1 males have produced chicks in approximately the ratio 2 black: 1 Columbian: 1 buff; again showing equality between the extended and restricted classes. The F_1 black females crossed with buff males have produced equal numbers of black and buff chicks, while the F_1 Birchen females have produced, when crossed with buff males, black, Columbian and buff chicks in a ratio approximating 2: 1: 1. The ratios as quoted above have all been obtained and will be reported in full when the adult classifications have been completed. All of these crosses represent matings of fowls heterozygous in extension ($E^m e^m$) with fowls recessive in extension ($e^m e^m$). The expectation is equal numbers of black (extended) and non-black (restricted) chicks. The experimental numbers at present are 99 black (E^m): 98 non-black (Columbian or buff e^m). A clear monohybrid segregation is evident between extension (E^m) and restriction (e^m) of black pigment.

The above results are all explained on the following hypotheses:

1. The black fowls have the dominant allelomorph of an autosomal gene (E^m) which determines the extension of black pigment to all parts of the plumage. The recessive allelomorph (e^m) of this gene is present in the Columbian and buff fowls.

2. The black fowls contain the recessive allelomorph of the dominant sex-linked gene *S* (silver). This dominant gene inhibits the production of buff ground color and causes the production of silver or white ground color;[1,2] it is known to be present in Columbian fowls, while the recessive allelomorph characterizes the buffs. As regards these two genes the blacks used in these experiments have proved to be $E^m E^m ss$ in composition, the Columbians $e^m e^m SS$ (male) or $e^m e^m S$- (female), and the

[1] Sturtevant, A. H., 1912, *Jour. Exp. Zool.*, 12: 499–518.

[2] Dunn, L. C., 1922, AMER. NAT., 56: 242–255.

buffs $e^m e^m ss$. Blacks are therefore genetically buffs with an epistatic gene for the complete extension of black pigment.

3. Extension of black is incompletely epistatic over silver so that in fowls of the genotype $E^m e^m Ss$ (male) or $E^m e^m S$-(female) silver appears in certain parts of the plumage, producing a pattern like that of the Dark Brahma.

Collateral evidence indicates that the gene for extension of black pigment (or one with similar effects) is present in Barred Plymouth Rocks and White Plymouth Rocks (as a cryptomere); and that it is absent in Columbian and buff varieties and in Rhode Island Reds.

Hurst[3] was probably dealing with the same gene in his crosses between Black Hamburgs and Buff Cochins, since F_1 from this cross consisted of all black chicks, while in F_2 black and buff chicks occurred in the proportions of 88 black: 31 buff. In interpreting the results of this cross Morgan[4] states that either one or two pairs of factors may be involved; which is right "could only be determined by an F_2 ratio." Yet the F_2 ratio is given by Hurst (p. 138) and is surely a sufficiently close approach to a monohybrid ratio. The ratios obtained in our experiments agree throughout with a mono-factorial interpretation.

It is believed that this gene will be found to characterize many color varieties of fowls in which black, either as a self color or as a component of a pattern extends to all or nearly all of the plumage. Concerning its origin no direct evidence can be offered at present. Its occurrence as a discrete unit indicates, however, that its origin was discontinuous and that black varieties probably had their genesis in mutation rather than in selection of particolored types toward black.

L. C. DUNN

THE EFFECTS OF SO-CALLED CONJUGATION IN SHELLED RHIZOPODS[1]

THE phenomenon of conjugation in the Protozoa is regarded as the forerunner of sexual reproduction in the higher animals,

[3] Hurst, C. C., 1905, Reports to the Evo. Comm., II, pp. 138–39.

[4] Morgan, T. H., 1919, Publ. Carnegie Inst. No. 285, p. 24.

[1] The experimental work on this problem was carried on in the Zoological Laboratory of the Johns Hopkins University. I wish to thank Dr. H. S. Jennings, of that institution, for suggesting the problem and for his aid in pursuing the investigations.

in which there is a union of a sperm and an egg. This latter process is fundamental in the life of at least all the higher Metazoa. By this union the race is perpetuated and hereditary characters are intermingled. It would seem that studies of a similar process in the unicellular forms might throw light upon the basic relations of the sexual process to the life of protoplasm.

Various investigators have shown that conjugation is a relatively common occurrence among the more complex Protozoa, such as the Paramecium, and that hereditary characters are intermingled in this way. During conjugation two Paramecia fuse by their oral surfaces and there is an interchange of nuclear material between the individuals. The latter then separate and reproduction by division occurs. The races resulting from such divisions show the effects of modifications due to hereditary characters coming from both the conjugating individuals.

In the case of the more primitive Protozoa, the Amœba and other Rhizopods, not a great deal is known. It has been observed that sometimes two individuals unite, but it is uncertain whether or not true conjugation occurs with interchange of nuclear material and subsequent modification of the offspring. If such proves to be the case, we shall have shown that the phenomena of sex are found in the very lowest animals, and are of general fundamental importance in the life process.

The present investigation was undertaken in the effort to throw some light on this question. An attempt was made to test the matter by inducing conjugation between various individual Rhizopods and noting if any inherited differences arose from these unions. (A cytological study of the behavior of the nuclei of such individuals must be made before the evidence can be fully weighed.) A shelled Rhizopod, *Difflugia corona*, was used, since the shell exhibits marked characteristics which vary among the different races of the species. The ordinary shellless Amœba presents so few characters of a permanent nature that it is unsuited for a study such as this. The Difflugia is an Amœba which builds from microscopic sand grains a shell shaped much like an old-fashioned soap kettle with the legs represented by spines projecting from the rounded aboral surface. The animal lives inside this shell and thrusts its pseudopodia from the oral opening. It reproduces by division, as other Protozoa, half the body being extruded from the oral

opening of the shell and a new shell being formed over the exposed part of the body, the two shells, old and new, lying mouth to mouth. The body then divides and the new individual moves away to lead a separate existence.

At times two Difflugias have been observed attached mouth to mouth and have been thought to be conjugating. When dividing the new shell is much lighter in color than the old and in the cases supposed to be conjugating both shells were of the same shade, that is both dark, so that the phenomenon did not seem to be that of division. It was this attachment or so-called conjugation which I endeavored to investigate.

It was found by Dr. Jennings (not published as far as I am aware) that two Difflugias could sometimes be made to attach themselves together by keeping them in a drop of distilled water for a few hours. At his suggestion, I used this method, endeavoring to hasten the process somewhat by pushing the individuals together by means of a fine glass rod. In several cases, the members of the pair became quite firmly united and remained so for some time. These pairs I then put into a drop of culture medium in the concavity of a hollow ground slide. In some cases such individuals never separated but died. In several instances they did separate and lived and began to reproduce by division.

Briefly, my procedure then was this. The number of spines varies in different races of Difflugia, sometimes averaging 2 or 3, sometimes as high as 5 or 6. The size of the shell also varies. I used the diameter across the widest part of the shell. I selected, for example, a small individual with a few spines and kept it under observation on a hollow ground slide in culture medium until it had produced an offspring by division. (The culture medium consisted of tap water containing the microscopic débris washed from the leaves of Elodea.) I then isolated the offspring and allowed it to produce a race, keeping each individual separate on a slide, and measuring and counting the number of spines of each. The original Difflugia I tried to mate, so to speak, with a large Difflugia with many spines, which had previously produced an offspring which I had isolated and allowed to found a race. If the large and small Difflugias became attached, supposedly conjugating, and were successfully separated, I then isolated each one on a slide and allowed each to start a line of progeny. I then had 4 lines going, one from each Difflugia before conjugation and one from

each after conjugation with the other. The following diagrams may serve to make my meaning clear.

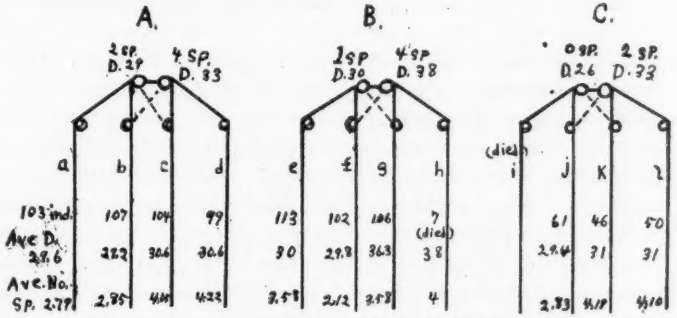

FIG. 1. Diagrams illustrating the manipulation of Difflugia in the experiments with so-called conjugation and the results attained in three cases. The two circles at the top of each diagram represent the individual Difflugias which became attached to each other. D., diameter- sp., spines. ind., individuals. Ave., average.

In each figure the circles at the top represent the Difflugia which were paired. For example, in *A* an individual with a diameter of 29 units founded a line *a* and then was paired with a Difflugia with 4 spines with a diameter of 33. This had previously started line *d*. After the separation of the Difflugias, they founded lines *b* and *c* respectively. The dotted cross lines represent the influence which might be expected to come from the other member of the pair if there were actual conjugation. Line *a* was carried on until 103 individuals were produced, the average number of spines being 2.79 and the diameter 29.6 units. Line *b*, started after the attachment of the progenitor to the other Difflugia, was carried for 107 individuals, with an average of 2.85 spines and a diameter of 29.2. The diagrams show the results gained from carrying out the lines in the other experiments. Unfortunately line *i* died before producing any individuals and line *h* produced only 7 before dying out.

By comparing the four lines in each experiment with each other, evidence may be gained relative to whether or not, during the supposed conjugation, the Difflugias exerted an effect upon each other sufficient to cause modification of the diameter and number of spines of the offspring in the direction of the line started by each before the attachment. That is, for example, line *b* should be more like line *d* than is line *a*, and line *c* should be more like *a* than is line *d*, and so on throughout the experiments.

A study of the data set forth in the diagrams will show that there is practically no modification of any line springing from a Difflugia, after the supposed conjugation, in the direction of the characters of the line founded by the other member of the pair before the union. Any slight changes seeming to show such modification are offset by just as marked variations away from that line. Comparing line *g* with *h*, it would seem that there is a marked modification of *g* in the direction of line *e*. It must be noted, however, that line *h* consists of only 7 individuals, so that the average is untrustworthy. In no other case is there any significant leaning toward the other line, although there are very slight tendencies in that way in the spine numbers in experiment A. Line *a* has 2.79 spines, *b* 2.85, *c* 4.05 and *d* 4.22. On the other hand, in experiment B the spine number in line *e* is 3.58 and in *f* only 2.12, to be compared with 4 in line *h*. It would be expected that line *f* would show a greater number of spines than line *e*. In experiment C line *k* shows a slight increase in spine number instead of the expected decrease. In general there are no apparent modifications of the offspring as the result of the pairing.

The experiments are open, of course, to the criticism that the attachment between the Difflugias was brought about by keeping the individuals in distilled water to bring them to the state of partial starvation which seems usually to be the forerunner of conjugation in the Protozoa. It is possible that under these somewhat unnatural conditions, the preliminary steps incident to conjugation would be inaugurated but that the process would stop before completion. In answer to this, I can only state that the Difflugias remained attached from 12 to 24 hours, thus allowing sufficient time for nuclear changes to have occurred. They became separated only when placed in cultures containing food.

To summarize then, as far as it is safe to base conclusions on the results of this limited number of experiments, it seems that the offspring of the Difflugias were not influenced by the attachment or so-called conjugation of the parents. From this fact it appears probable that this phenomenon of attachment sometimes observed in the shelled Rhizopods is not a true conjugation and that there is no interchange of nuclear material between the individuals taking part in it.

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ON REARING SEXUALLY MATURE PLATYNEREIS MEGALOPS FROM EGGS

I

THE literature affords so few cases of marine animals reared under laboratory conditions that the writer ventures to communicate his successful attempts to carry through to sexual maturity the nereid, *Platynereis megalops,* from eggs laid in the laboratory. This work had its origin in a suggestion made by Dr. F. R. Lillie in 1911 that the capacity for cross fertilization between *Nereis limbata* and *Platynereis megalops* be tested. At that time, however, since we knew so little of the life history of these forms, we felt that it was necessary to get all data possible on each life history in order to have a standard of comparison for the life history of the hybrids. So far all efforts to cross these nereids have failed. The difference in the breeding habits of *Nereis* and *Platynereis* is so striking that this alone might account for the failure of cross fertilization. *Nereis* sheds eggs into the sea-water where fertilization takes place; *Platynereis* lays inseminated eggs soon after copulation. However, this very difference is calculated to enhance the interest attaching to the cross fertilization. It might be possible to study the inheritance of the egg-laying reactions. In addition, early observations revealed that the young *Platynereis megalops* closely resemble *Nereis dumerilii.* Since, as is well known, *Nereis dumerilii* has a complex life history, we felt that the life history of *Platynereis* might well repay study for its own sake.

II

PLATYNEREIS MEGALOPS REARED UNDER LABORATORY CONDITIONS TO SEXUAL MATURITY

The writer has found that it is possible to rear *Platynereis megalops* to sexual maturity under laboratory conditions. This was first accomplished in 1913–1914, repeated in 1920–1921, and again in 1921–1922. The results may be briefly recounted.

Methods

Males and females caught with a hand net in the evenings of the July and the August full moon are kept in separate dishes. In the laboratory as shortly after capture as possible a male and

a female are placed in a finger bowl of clean sea-water. After copulation and egg-laying the animals are removed from the finger bowl. After the jelly has been extruded by the eggs, the supernatant sea-water is poured off, leaving the eggs in the mat of jelly stuck to bottom of the bowl. At first cleavage, which is invariably one hundred per cent., the jelly-mass is gently broken up and the eggs equally distributed among seven to ten finger bowls of clean sea-water. Early the next morning the sea-water is changed. At this time all eggs that possess fewer or more than four oil drops, one in each macromere, are discarded. Only those larvæ that possess four oil drops evenly distributed among the four macromeres give rise to normal swimming larvæ. As the trochophores rise to the surface in each dish they are pipetted off. Trochophores that fail to swim at the surface in twenty-four hours lack the viability of those that rise earlier.

The young larvæ are kept in subdued light a few in each dish because they tend to aggregate in such dense masses that many die off. This tendency to collect in one spot makes it easy to change the water and thus avoid too great rise in temperature, which is fatal to the animals. The larvæ will reach the stage of three swimming segments without the addition of food.

When the segments appear, the larvæ must now be watched very carefully in order that food may be given at the proper time. *The criterion for the initial feeding is the complete disappearance of the oil drops from the entoderm cells.*

In the eggs of both *Nereis* and *Platynereis* there is at the time of fertilization a girdle of some eighteen to twenty-two oil drops in the equatorial zone. These oil drops in the maturation stages following insemination move to the vegetative pole. During cleavage the number of oil drops is reduced to four large globules which normally are distributed to the cells of the gut. Beginning with the third or fourth day after laying, the oil in the gut cells of the larvæ begins to form an emulsion of smaller and smaller drops. It is thus possible to follow the history of the oil drops very fully in these creatures that make veritable living test tubes in a fat-digestion experiment. If food is given the worms before the oil has been completely used, they are killed in large numbers. On the other hand, food must not be withheld too long after the disappearance of the oil. The first feeding consists of ten c.c. of a diatom culture known by previous examination under the microscope to be free of metazoa or larvæ,

strained through three thicknesses of bolting silk of very fine mesh. As the larvæ add segments more food is given. When the larvæ build their tubes both food and mud are added until the bottom of the dish is well covered. The method of preparing the diatom culture may now be considered.

In 1911 I procured food according to the method described by Hempelmann in his study of the life history of *Nereis dumerilii* at Naples. This method consists principally in scraping the growths from the live tables. The bottom and sides of aquaria under running sea-water frequently show a felt-like growth of diatoms and protozoa. Scrapings from such aquaria suspended in sea-water will give food sufficient to keep a few young worms of both *Nereis limbata* and *Platynereis*. The method does not allow the rearing of any large number of worms. Similarly, attempts at a pure culture of diatoms gave poor results.

In 1913 through the kindness of Dr. Caswell Grave I procured a remarkably fine culture of diatoms from Beaufort, N. C. With this, the first sexually mature worms were obtained. But obviously, *Platynereis* at Woods Hole must live on food got in Woods Hole waters. I, therefore, made various attempts to get an adequate diatom culture from the immediate vicinity. The successful method follows.

At the beginning of the season mud is taken from Eel Pond, near-by flats, or scraped from eel grass, together with animal and plant life. This is placed in jars with the addition of an equal volume of sea-water. The jars are then covered with glass plates and set aside in subdued light. In a day or so all metazoa—worms, crustacea, ascidians, etc.—are dead. After a period of putrefaction, the culture purifies itself and a rich growth of diatoms begins.

For young worms a suspension of diatoms strained through several thicknesses of bolting silk is used. The diatoms for this purpose are previously examined under the microscope, one c.c. at a time; usually no metazoa are found. The suspension is then made up in filtered sea-water. As the larvæ increase in size and vigor food is added in greater quantities.

A brief summary of the three cultures of *Platynereis megalops* reared to sexual maturity may now be given.

The Larval Cultures

The 1911 and 1912 larvæ were not kept after September first.

The 1913 Culture.—During August, 1913, from eggs laid in

the laboratory by twenty females over 100,000 larvæ were reared. On the eve of my departure from Woods Hole the larvæ were all carefully removed from their tubes and placed in half-gallon Mason jars. Each jar contained 500 c.c. of the rich Beaufort diatom culture and sea-water to within ten centimeters of the top. The jars were then tightly covered and set aside. After the worms had had time to build new tubes, the jars were shipped to Washington, D. C. The worms at this time averaged about 10 mm. in length. Early in June, 1914, these worms were shipped from Washington to Woods Hole. Relatively few survived the journey. The largest worms (females), brought carefully in a hand bag, died before the journey was half made. Since during the winter hundreds of these worms had been killed periodically for future study, the number left from the 1913 culture had been greatly reduced. Some animals of this culture were carried through the summer of 1914. They never reached sexual maturity. They were taken back to Washington at the end of 1914. In 1915 they were brought back to Woods Hole and returned to Washington that fall. During this period they still showed no change.

In Washington the animals were kept in the clamped jars without any change of water or additions of distilled water. One culture was kept in a battery jar covered with a glass plate. Nor was any addition ever made to this culture. The jars were kept at room temperature in subdued light. To avoid contamination worms removed for study were from the culture in the battery jar only. After observing the worm I never replaced it, but killed it for *in toto* mount or sectioning.

In 1917 several very fine cultures of worms were started, but they died in transit to Washington. In 1918 and 1919 no worms were reared.

The 1920 Culture.—In August, 1920, very beautiful cultures of about 50,000 larvæ were started: unfortunately, the majority of these died very suddenly late in August. About a thousand worms survived. These were distributed among twelve dishes with food and left over winter in the heated laboratory at Woods Hole. The dishes were left covered with glass plates exposed to north light. No change was made in the water or any additional food given during the period September 1, 1920, to June 1, 1921. On May 17, 1921, about 200 worms of different sizes were found in the dishes. Of these some were preserved from time to time.

The history of the others shows that the first female with ripe eggs appeared June 5. She was discovered slowly crawling around the dish near the surface of the water. In color and in form this animal resembled the females collected during the breeding season. In size she was rather below the average and somewhat more sluggish. The eggs in size, color and form were identical with the eggs got from animals captured during the breeding season. Subsequently, mature females were found at intervals through the summer. No males appeared until late in June. Eventually, thirty-two females and twelve males fully mature were got from this 1920 culture.

Males got from the sea copulate with females reared in the laboratory; such females lay normal eggs that give rise to larvæ of a high degree of viability. Males reared in the laboratory copulate with females taken from the sea. The eggs are perfectly normal. On only one occasion did I find a male and female from this 1920 culture sexually mature at the same time. They copulated in normal fashion. The eggs laid were normal in every respect and gave rise to larvæ that I kept for two weeks before discarding. These larvæ could not be distinguished from larvæ resulting from eggs laid by animals taken from the sea.

The 1921 Culture.—At this writing only two mature animals (females) have appeared—one May 1 and one May 6, 1922.[1]

The Rate of Growth

Some idea of the rate of growth in these worms may be obtained from data collected from the 1913 culture. This was the only culture on which I had the opportunity to make continuous observations.

Life History

Observations so far made on cultures of *Platynereis megalops* reared in the laboratory from eggs laid by animals taken from the sea do not reveal any indication of a sexually mature intermediate form. So far, all eggs obtained appear to be identical with those got from animals in nature. This would seem to suggest that the life history of *Platynereis* is simple—without the complexity of form and sexual condition found in *Nereis dumerilii*, which *Platynereis* so closely resembles. It must be clearly

[1] Since the above was written, 23 animals have reached maturity—17 females and 5 males. One reason for this sex ratio is that the males have difficulty in getting out of their tubes; their mortality is therefore high.

stated, however, that on this point the observations so far made are not conclusive. In order to determine fully that the eggs laid by worms in the cultures in the laboratory are from the same worms started in the culture and not from an intermediate form and are the only eggs laid, it would be necessary to make continuous observations on isolated worms. So far it has not been feasible to do this, since it would mean practically continuous residence at Woods Hole through the winter.

TABLE I

RATE OF GROWTH OF PLATYNEREIS MEGALOPS FROM EGGS LAID ON THE EVENING OF JULY 21, 1913

Date	No. of Segments with Parapodia	Length in Mm.
July 28, 1913	3	
July 29	4 to 5	
July 30	5	
July 31	6	
August 2	10	
August 6	16	2
August 8	22	4
August 12	26	5
August 26		7
Sept. 15		14
Oct. 1		18
Nov. 1		20
Dec. 1		25
Jan. 10, 1914		30
Feb. 2		33
March 1		40–45
April 3		40–50
May 6		50–60
May 28		40–50

On the other hand, it is just barely possible that in a state of nature the life history is more complex than in the laboratory cultures. Under operation of changes in such factors as density of the sea-water, food, and temperature, the life history of the worms may be modified. That this possibility deserves some consideration we may conclude from the sex ratio, if such meagre data will allow. In the laboratory cultures females appeared first in all three years and they outnumber the males. In nature just the reverse is true.

Whatever our conclusions as to the interpretation of these observations, it seems to the writer that the life history of this interesting nereid is worthy of further study.

A Comparison with Other Forms

The method used for rearing sexually mature *Platynereis* from the fertilized egg has been used to rear other worms through to the adult stage: namely, *Pectinaria gouldii, Diopatra, Nereis limbata,* and *Chætopterus.* In all cases the worms were reared from eggs cut out of the females and inseminated in sea-water. In no case were the worms kept beyond September 15 (from one to three months). Though it is usually stated that artificial insemination of *Diopatra* eggs is not possible, every attempt made by the writer in 1911, 1912, 1913, 1914, and 1915 was successful. There is one danger to avoid with these eggs—initiation of development by mechanical shock. The worms reared from *Diopatra* eggs are if anything more hardy than those of *Platynereis.* In 1913 I reared *Diopatra* in a watch glass to a length of four centimeters.

Pectinaria gouldii are likewise readily reared from eggs inseminated in the laboratory. These eggs are extremely beautiful, small, and almost wholly transparent. They are easy to handle. I have found them the best eggs in my experience for study under high power (oil immersion lenses).

The specimens used were from the Eel Pond and are normally smaller than *Pectinaria* found outside of Eel Pond. They are infested with a distome and an interesting ciliate; the latter I did not find in the larger specimens (1911). This, if it be generally true, together with the size of the Eel Pond specimens makes an interesting case from the point of view of ecology.

Among the shed spermatozoa of *Pectinaria* are many in bundles that break up after a short time in the sea-water. In addition to these one can always get bundles of spermatocytes, immature sperm, etc., by puncturing the body wall. It is a very excellent form to use for the study of cytoplasmic inclusions: it is possible to get the whole history of the sperm on one slide.

My object in studying these ova was to try to learn if size, opacity, and yolk influence the ease with which the animals can be reared under laboratory conditions. I found no correlation. Thus, the egg of *Platynereis* is almost transparent; it measures 180–200 μ. *Nereis* egg has more color and measures about 100 μ. The *Nereis* egg is the hardest of all to carry through. The egg of *Pectinaria* is small and almost wholly transparent. It is readily reared. The *Chætopterus* egg has more color than that of *Nereis* and is smaller. It is easier to rear than the egg of *Pectinaria.* The *Diopatra* egg is wholly opaque; it is the largest

of the five eggs and perhaps the easiest to rear. The eggs may be arranged according to size, depth of color and ease with which they may be reared as follows:

Size	Depth of Color	Ease of Rearing
Diopatra	*Diopatra*	*Diopatra*
Platynereis	*Chætopterus*	*Platynereis*
Nereis	*Nereis*	*Chætopterus*
Chætopterus	*Platynereis*	*Pectinaria*
Pectinaria	*Pectinaria*	*Nereis*

As in the case of *Platynereis* the essential point in rearing these annelids is to give them food at just the right time in the larval stage. This time varies somewhat with each form. Briefly, food must not be given before the yolk and oil are wholly used up. One needs but to watch the larvæ, note the disappearance of the oil from the gut, and then add diatoms.

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AN OBSERVATION ON THE "CLUSTER-FORMATION" OF THE SPERMS OF CHITON[1]

WHILE engaged with an inquiry into the natural history of the chitons, in 1918,[2] I several times made an observation which may have a bearing on the significance of sperm-clusters, and on the mechanism of their formation. The matter could not at the time be adequately investigated, but since I shall not soon be in a position to examine it further my observations are here related for what they may be worth. The species concerned is *Chiton tuberculatus* Linn., an intertidal form quite abundant at Bermuda. It is necessary to note, first, certain features of the breeding process, which seems to me to have heretofore been

[1] Contributions from the Bermuda Biological Station for Research. No. 119.

[2] The corrected proof and manuscript of this article were returned to the publisher Aug. 18, 1920; but the corrected article was accidentally taken out of type in the office of the printers. The author has now rewritten the paper. E. L. M.

somewhat misunderstood. In another connection I shall describe several aspects of the reproductive activities of these animals, the present remarks having to do merely with the act of fecundation.

Although it has commonly been held that the liberation of eggs by a female chiton is due to the reception of spermatic fluid diffusing into her respiratory water-currents from a nearby male, the process of fertilization would appear in fact to be initiated in a quite different manner. Stated briefly, the presence of one or more neighboring females serves in some way to activate the discharge of sperm by the males, the spermatic substances secondarily inducing the liberation of eggs. Normally this occurs only at those periods when the flow of the tide begins just before sunrise, the shedding of the genital products commencing as the chitons become covered by the sea. The discharge of sperm can, however, be induced artificially at certain times, in the laboratory, even a month or more before the eggs are matured.[3] A method which several times yielded this result consisted in keeping some male chitons in a damp, darkened vessel for about 14 hours, then covering them with sea water and admitting light. It should be noted here that *C. tuberculatus* is an animal nicely fitted for observations of this kind, because the differential pinkish tint of the soft tissues of the females permits the quick and accurate identification of sex.[4]

In May, a month before ripe eggs are seen, it was noticed that when sperm diffusing from a male, in a glass dish, was taken up between the ctenidia of a female, it issued from the posterior ends of the ctenidial channels in an altered state, for the sperm-stream was then seen to contain numerous agglutinated masses of active sperms, which persisted in sea water for at least half an hour.

During natural fecundation, however, no sperm-balls are formed. The thick glutinous stream of spermatozoa passes under the girdle of a female, is somewhat diluted with sea water

[3] That the discharge of sperm is under nervous control is indicated by the behavior of male *Chætopleura* following strychninization (cf. Crozier, 1920, *Jour. Gen. Physiol.*, Vol. 2, pp. 627–634).

[4] See Crozier, W. J., 1920, "Sex-correlated Coloration in Chiton," AMER. NAT., Vol. 54, pp. 84–88. Tidal, or rather lunar, periodicity in the liberation of gametes has been observed also in *Chætopleura;* I was able to note a probable lunar periodicity in this genus, in 1919, at Woods Hole, and the point is dealt with at length in a recent paper by Grave, B. H., 1922. *Biol. Bull.*, Vol. 42, pp. 234–256.

by the tractive current, and emerges posteriorly in company with numerous large greenish eggs, about which, under the microscope, it can be seen that many sperms are gathered. But no real "cluster-formation" takes place.

The body juices of the ripe female, whether or not diluted with sea water, do not cause agglutination of sperm suspensions. But ovarian extracts from (*mature*) eggs in sea water do induce decided and apparently typical agglutination. So far as I know, sperm-agglutination by ovarian extracts has not previously been seen in molluscs.[5] Sea water into which ripe eggs have been shaken from an ovary and the whole allowed to stand for half an hour has a similar agglutinative effect.

Concerning the significance of the cluster formation, then, these two points seem significant: (1) the absence of such a process in normal fecundation, and (2) its conspicuous occurrence when sperm, before the real onset of the breeding season, has passed through the ctenidial channels of males or immature females. It could not be discovered whether or not the *mature* female in a non-spawning interval would cause this cluster production, because at such times the consistent response of a female to an impinging current of sperms was to depress the girdle to the substratum, thus cutting off the water current carrying sperms, and, by reducing the volume of the ctenidial channel, violently to expel from below the sperms already admitted.

These observations do not, of course, permit analysis of the rôle of egg-substances in fertilization of chiton, but do serve to point the contention that mere evidence of sperm agglutination (cluster formation) may well have no bearing on such analysis. It is possible that the sperms set free at a period before the natural ripening of eggs are in some degree immature, their surface perhaps more sticky, or liable to be made so by slight external changes experienced in passing between the gill filaments of another individual.

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[5] Loeb, J., 1916, "The Organism as a Whole," x + 379 pp., New York.
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